Carotid and Cerebral Arteriosclerosis in the Rat
By Bernard C. Wexler, Ph.D., and Charles W. Truo, M.D.
With the technical assistance of Nancy Seamer and Dilman Conatser

The importance of cerebral vascular disease is well known as is its increasing frequency in all age groups. Progress in the clinical management of cerebrovascular disease has stemmed from increasing appreciation of the significance of deranged function in the arteries of the head and neck. There is a growing realization that the cerebral arteries may have a different physiological and morphological pattern of response than other branches of the aorta. It has been recognized for some time that cerebral infarction or allied cerebrovascular diseases may best be correlated with arteriosclerosis in the cervical and vertebral arteries rather than with cerebral arteries proximal to the damaged brain tissue.

Very little information has been gained from experimental investigation because the rabbit, and other species most commonly used for the study of arteriosclerosis differ from man in that they seldom develop arteriosclerosis above the level of the aortic arch. During the study of the development of spontaneous arteriosclerosis in male and female breeder rats of several strains, it was found that the carotid arteries were frequently the site of marked arteriosclerotic lesions. Therefore, a more precise study was made of the arterial lesions in the carotid and cerebral vessels. Because degenerative changes in nervous tissue are most frequently due to vascular alterations, neuropathological observations were also made of the brain tissue in these animals.

Methods
Male and female breeder rats of several strains develop arteriosclerosis spontaneously. The incidence and degree of arteriosclerosis varies from strain to strain. When arteriosclerosis of the systemic aorta is severe, the arteries of the neck frequently also show severe arteriosclerosis. Severe arteriosclerosis of the carotid arteries has been observed in Lewis, Long-Evans, Holtzman and Sprague-Dawley rats. In order to investigate the nature of the cerebral and cervical arteriosclerosis in breeder rats more precisely, we made a special study of Sprague-Dawley breeder rats of the Sprague-Dawley Farms. Male and female discarded breeder rats of the Sprague-Dawley Farms have a consistently high incidence of arteriosclerosis. A description of the morphology of these lesions has been published. Further details of the distribution and types of lesions found in the Sprague-Dawley strain, and in other strains as well, will be published soon.

Our experience to date has been that male breeders develop arterial lesions which remain microscopic. However, the female breeders develop varying degrees of arteriosclerosis which may be easily visualized by gross inspection. Thus, arteriosclerosis in the female breeder lends itself readily to evaluation at time of autopsy. The degree of gross arteriosclerosis in female breeders has been arbitrarily divided as follows: none, minimal, moderate and severe. The details of this scoring system will be published.

In this particular study, virgin and discarded breeder rats of the Sprague-Dawley strain were used exclusively. Female breeders were sacrificed and their aortae were classified according to the four categories described above. The status of these animals were collected so that there was a minimum of six aorta for each of the categories of gross arteriosclerosis, i.e., none (6 samples), minimal (6 samples), moderate (6 samples) and severe (6 samples). Six male discarded breeders, as well as six virgin female and six virgin males, comparable in age to the breeders, were also sacrificed. All of the animals were housed in six-conditioned quarters upon receipt and until
FIGURE 1
Carotid artery. An early lesion consisting of focal subintimal mucoid deposition (see raised pale area in photo), increased nuclear basophilia, medial ground substance swelling and beginning disruption of medial elastic lamellae. H and E, × 150.

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RESULTS
A. Carotid Arteries. In this study, as in all previous studies on the arteriosclerosis found in breeder rats, the abdominal aorta was the first site to show arteriosclerotic changes. The abdominal aortae in both male and female breeders which were classified as "none" on gross inspection contained microscopic lesions. No changes could be found in the carotid arteries until the sclerosis had first spread to the arch of the aorta from the abdominal aorta. This applied to both male and female breeders.

The first changes which occurred in the carotid artery followed the same pattern as in the abdominal aorta. Those sites which later became arteriosclerotic showed first increased basophilia of all nuclear elements in the tunica media (fig. 1). Fibroblasts or mesenchymal elements increased in number in these foci, accompanied by ground substance swelling. Immediately subjacent to these swollen sites the intima showed increased metachromasia or Hale stain by guest on August 29, 2017 http://circres.ahajournals.org/ Downloaded from
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FIGURE 2
The same intimal lesion shown in figure 1 stained with the Hale stain. The pale-staining intimal mucoid material shown in figure 1 is composed of acid mucopolysaccharides (deep black in photo) at the base of the arterial lesion and is capped by fibrous material (light grey in photo). Hale stain, X 150.

positive (fig. 2). This material is mucopolysaccharide. The sites of heavy intimal mucopolysaccharide accumulation in the carotid artery did not show as much collagen deposition as is found in mucopolysaccharide pools in the abdominal aorta. In the case of the male breeder, deeply basophilic mesenchymal cells invaded the intima. The mesenchymal cells enlarged and resembled cartilaginous cells. The sites became strongly metachromatic and contained marked amounts of mucopolysaccharide. These early changes began in the lower portions of the carotid artery and spread toward the cerebrum as the arteriosclerosis progressed (fig. 3).

Thromboses, aneurysms and the spectrum of arterial defects seen in the systemic aorta of breeder rats were not found in the carotid arteries. Elastosis and medial dystrophic calcification of the lower portion of the carotid were much more prevalent in the female breeder. Calcification and bone formation, however, were found preferentially in the carotid arteries and the arch of the aorta (fig. 4). These lesions stemmed from the mesenchymal cell infiltration which occurred in the intima and media (fig. 5). The process was particularly evident from the point of bifurcation of the carotid down to and including the arch of the aorta. Cartilaginous metaplasia was frequent in the male breeder but it reached much more advanced stages of
Carotid artery of male breeder showing rounded, giant-sized basophilic cells which were formerly flattened mesenchymal cells. The halo of basophilic material (black in photo) surrounding these cells is intensely metachromatic and is believed to be mucopolysaccharide being actively secreted by these cells. H and E, X 150.

Marked elastic tissue disruption and scarring which accompanies the cartilaginous metaplasia in a carotid artery. Elastic fibers appear black, cartilaginous material intense black. Note marked disruption of ground substance (see arrows). Gomori’s elastic tissue stain, X 50.

In the smaller branches of the carotid, e.g., thymic (fig. 7) and sublingual arteries, the lesions showed distortion of the normal contour of the elastica and intimal accumulation of a basophilic material. None of the male and female virgin rats showed any gross or microscopic evidence of cerebral or systemic arteriosclerosis.

B. Intracranial Arteries. Vascular alterations in the intracranial arteries were detectable especially in those cases where the carotid artery showed extensive arteriosclerotic involvement. The vascular changes were not prevalent and were not as severe as those found in the carotid arteries. Several types of alterations were encountered.

In the larger intracerebral arteries the internal elastic membrane was rather prominent relative to the thin wall of these arteries. The elastica often showed aneurysm-like dilations in several foci and invariably the outpouching faced the lumen (figs. 8 and 9). This was by far the most frequent type of lesion encountered.

Some of the larger intracerebral arteries also showed an unusual medial vacuolization.
and palisading of the smooth muscle cells of the media as well as the presence of giant-sized smooth muscle cells. Other arteries of similar size occasionally were found with small intimal hillocks which stained intensely with the Hale stain (fig. 8). It may be added that the smaller cerebral arteries, in some instances, showed medial thickening with accumulation of a Hale positive material which was also metachromatic with toluidine blue. However, this type of lesion was infrequent.

C. Nervous Tissue. Grossly, there was no evidence of cerebral damage in any of the brains examined, even in brain tissue from animals showing severe degrees of carotid arteriosclerosis.

Microscopically, rat brains show a comparatively rudimentary cerebral cortex with surprisingly few penetrating vessels. The most superficial surface of the cortex is composed of a single layer of glial cells which does not seem to follow the penetrating vessels into the brain substance. In the present study no significant changes could be found in glial tissue. In a few instances only, in animals with moderate or severe arteriosclerosis, one could observe a dropping out of neurons and clusters of ghost cells or anuclear nerve elements. However, this occurred so infrequently that it could be ascribed to natural cyclic degeneration as a part of the aging process rather than to any specific vascular impairment. Occasional foci showed cells which were hyperchromatic or extremely basophilic with loss of nuclei or nuclear detail (fig. 10). In these instances, oligodendroglial elements were found filling the defect. The few lesions found were located mostly in the middle and posterior portions of the brain.

Discussion

It has been shown that arteriosclerosis in arteries external to the brain, particularly of
the carotid and vertebral arteries, is very common in cerebrovascular disease. Arteriosclerosis of the carotid artery appears to be its most frequent cause.\textsuperscript{11-15} Further, studies of the cerebral arteries indicate that this vascular bed is usually the last to be affected by arteriosclerosis.\textsuperscript{4, 11, 15} The development of arteriosclerosis in breeder rats of the Sprague-Dawley strain, and in other strains as well, follows the same course. Arteriosclerosis in breeder rats begins in the abdominal aorta, appears later in the coronary arteries, and in advanced cases is found in the carotid arteries.\textsuperscript{9}

It is also of interest that the lesions in the carotid arteries follow the same general histopathological pattern observed in the abdominal arteries with the early accumulation of subintimal mucoid substances. This is in agreement with the recent work of Baker and Iannone\textsuperscript{17-18} and the earlier work of Wallhoff.\textsuperscript{12} These investigators found that arteriosclerosis of the cerebral arteries was characterized by ground substance alterations, increased connective tissue deposition and little or no lipid accumulation. One of the prominent features found in the arteriosclerotic lesions of breeder rats of this strain, as well as in other strains, is the profound degeneration of elastic tissue and the predilection of the carotid arteries towards cartilaginous metaplasia and bone formation.\textsuperscript{9} A similar tendency of human carotid arteries toward elastic tissue degeneration was described by Baker and Iannone.\textsuperscript{17} However, they concluded that there was no difference between the sexes in the incidence and degree of cerebral arteriosclerosis. The rats in our study, on the other hand, showed a very definite sex difference in the degree of arteriosclerosis in the carotid arteries. The female breeder developed severe lesions characterized by elastosis, calcification and bone formation. The minimal arteriosclerosis found in male breeders may be due to the fact that the male breeder seldom lives long enough to develop advanced arteriosclerosis in any portion of the arterial tree.

The carotid arteries showed much mesenchymal cell proliferation, mucopolysaccharide deposition, cartilaginous metaplasia and bone formation. A satisfactory explanation for these observations is not immediately apparent. Perhaps these arteries are under unusual stress. Alterations in oxygen, sodium and potassium within connective tissue ground substance may be involved as they are known to have marked effects on the stage of polymerization of ground substance mucopolysaccharides, collagen formation and growth of cartilaginous tissue.\textsuperscript{15, 20} Investigations are now under way to determine possible biochemical differences between the carotid arteries and other segments of the systemic aorta.

It is pertinent to emphasize the fact that many branches of the carotid arteries showed evidence of generalized arteriosclerosis. For example, branches to the sublingual glands, thymus, thyroid and other structures in the neck showed subintimal mucoid deposits, elastic tissue degeneration and eventual fibrosis. Baker and Iannone described similar changes in human arteries of comparative caliber.\textsuperscript{21} The presence of ubiquitous arteriosclerosis in the arteries of the neck is stressed in light of the comparatively minor changes in the cerebral arteries discussed below.

It is rather remarkable that the intracranial arteries of the head showed comparatively minor involvement even in cases of severe fulfilling arteriosclerosis in the arteries of the neck. This termination of progressing severity of arteriosclerosis at the base of the skull indicates some protective mechanism which differs in these two closely related, but seemingly separate, vascular beds. A similar situation obtains in the human. The presence of parenchymatous degeneration of the brain can best be correlated with arteriosclerosis of the carotid arteries rather than with cerebral arteries most proximal to the site of damage.\textsuperscript{1, 5, 10-16} The significance of the polypoid outpouchings of the internal elastic of the intracranial arteries is difficult to interpret and is without counterpart in any of the other vascular beds.

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in the breeder rat. However, the association of mucopolysaccharide with elastic tissue degeneration in general has been a constant finding in these animals. Zugibe and Brown found mucopolysaccharides in human cerebral arteries but could not associate elastic tissue changes in these arteries with the presence of mucopolysaccharide. Zugibe and Brown found mucopolysaccharides in human cerebral arteries but could not associate elastic tissue changes in these arteries with the presence of mucopolysaccharide.

The loss of neurons, the presence of annular elements, and glosis was so slight in these animals that it would be difficult to assign any significance to their appearance, even in those cases of severe arteriosclerosis. However, the absence of brain tissue damage in these animals, despite marked vascular disorganization of the carotid arteries, is in accord with the findings of others in humans and primates, i.e., that intracranial circulation appears to be unaffected despite severe carotid arteriosclerosis. For example, Meyer and Denny-Brown have shown that monkeys do not develop cerebral infarction despite complete occlusion of the middle cerebral artery.

Comparison of these arterial lesions with those in other species is difficult because cerebral arteriosclerosis is seldom found in experimental animals. Despite the differences between rat and man, it is pertinent to note that there are several aspects of the morphological and temporal development of this disease in the two species which are quite similar. This would suggest that the rat might be useful in the study of the pathogenesis of cerebral arteriosclerosis.

Summary

Male and female breeder rats spontaneously develop arteriosclerosis. This occurs first in the aorta and coronary arteries and subsequently in the carotid and intracranial arteries. The carotid arteries show mesenchymal cell proliferation in the media followed by subintimal swelling and mucoid deposition, elastosis, cartilaginous metaplasia and eventual bone formation. Lipid deposition occurs infrequently, and then only in advanced lesions in the media. Severe lesions are much more frequent in the female breeder and the tendency towards cartilaginous metaplasia and bone formation is peculiar to the carotid arteries. The cerebral arteries show little arteriosclerotic involvement except for aneurysm-like outpouchings of the internal elastic membrane associated with mucopolysaccharide accumulation about the sites of elastic tissue disruption. Despite severe fulminating arteriosclerosis in the carotid arteries, the cerebral arteries and the brain tissue itself show little or no significant evidence of degenerative change. These results are interpreted to mean that the cerebral arteries of the breeder rat follow a different physiological and morphological pattern of response than the rest of the arterial system.

References

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