FOR SEVERAL YEARS we have been studying the spontaneous development of hyperglycemia, hyperlipidemia, hypertension, and arteriosclerosis in repeatedly-bred, male and female rats, e.g., Sprague-Dawley (S-D), Long-Evans, Wistar, etc. The incidence and severity of aortic lesions is greater in female when compared with male breeders and appears to be related to the copulatory activity or competitiveness of the males and the number of pregnancies and pups per litter in the female breeders. Our investigations strongly suggest that abnormal activity of the hypothalamic-pituitary-adrenal-gonadal axis associated with repeated breeding, conditions these animals toward a Cushing's disease-like spectrum of degenerative changes, e.g., hyperglycemia, hyperlipidemia, hypertension, and premature arteriosclerosis and aging.

In ancillary investigations, we subjected sexually mature male and female SH rats, whose hypertension was well-established, to repeated breeding. SHR breeders did not develop the extensive cardiovascular degenerative changes observed in all other normotensive strains of breeder rats. Instead, they manifested intimal hyalinosis and medial hypertrophy and hyperplasia of the small parenchymatous arteries of the testes and ovaries, exclusively. However, when repeatedly-bred SHR with hyalinosis of their gonadal arteries were subjected to surgical adrenal enucleation, athero-arteriosclerotic lesions appeared within their aortae identical to those observed in all other strains of breeder rats.

In order to probe further into the morphologic reaction of the arterial wall of SHR, we subjected normotensive and non-arteriosclerotic (virgin), and mildly hypertensive but arteriosclerotic (breeder) male and female S-D rats to carotid artery ligation injury. Similarly, severely hypertensive but non-arteriosclerotic (virgin) and severely hypertensive but arteriosclerotic male and female SHR were also subjected to carotid artery ligation injury. Segments of carotid arteries proximal and distal to the ligation site were examined histopathologically to determine the morphologic nature of the reaction of the carotid artery wall to a standard injury in the various host animals with and without hypertension or arterial disease.

Materials and Methods

Male and female, virgin and breeder, Sprague-Dawley (S-D) and spontaneously hypertensive rats (SHR), raised and bred in our Research Breeding Colony, were used. The SH rats were originally derived from the Okamoto: Aoki (Kyoto) strain and were kindly provided by Dr. C. Hansen of the National Institutes of Health. Except for the presence of hypertension in the SHR, both the virgin SD and SHR were healthy. Neither strain had arterial disease and both strains were comparable in age to the breeder rats. The male breeder S-D and SHR had been in active use since they were 90 days old and had sired 5 litters. The female S-D and SHR breeders had given birth to and suckled, in close succession, at least 4 to 5 litters. All of the animals were 6 to 9 months of age. The animals were housed in air-conditioned, humidity and light-controlled quarters and were fed a regular commercial rat chow (Rockland: Teklad) which has a relatively low fat content (4%) on an ad libitum basis.

When the female breeder rats had completed their fifth pregnancy and 28 days of lactation, both virgin and breeder rats were selected at random and segregated into 8 groups of 24 animals per group, i.e., male and female, virgin and breeder, S-D, and male and female, virgin and breeder, SHR. Under light Seconal anesthesia, a longitudinal incision (2 cm) was

SUMMARY The common carotid arteries of normotensive non-arteriosclerotic Sprague-Dawley (S-D) rats, mildly hypertensive but arteriosclerotic breeder S-D rats, severely hypertensive but non-arteriosclerotic virgin spontaneously hypertensive rats (SHR), and severely hypertensive breeder SHR were ligated to induce injury. Three weeks post-ligation, the animals were killed and histopathological sections of the ligated artery demonstrated myointimal proliferation without occlusion in the normotensive S-D rats but myointimal proliferation with occlusion in the severely hypertensive SHR. Breeder S-D rats with moderate hypertension manifested a high incidence of total occlusion by combined myointimal proliferation and thrombosis. Severely hypertensive breeder SHR manifested a high incidence of massive thrombi containing cholesterol clefts causing total occlusion of the injured artery. It is suggested that the severity of the hypertension and the hormonal-metabolic milieu conditions the morphologic response of the arterial wall to injury.


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made lateral to the midline of the neck over the hyoid musculature. Fascia and muscles were displaced to expose the triad of carotid artery, jugular vein, and vagus nerve. The carotid artery was carefully separated from this complex and a single ligature placed about the common carotid artery 2 cm below the bifurcation of the carotid artery into the external and internal carotid arteries. The ligature was tied snugly to occlude the vessel but not tight enough to cause tearing or damage. Muscle and skin layers were closed as separate layers. Just prior to surgery, the systolic blood pressure of each animal was recorded by means of the Friedman: Freed microphonic manometer and tail cuff, to establish the presence or absence of high blood pressure.

All of the animals were killed 3 weeks after carotid artery ligation. The occluded carotid arteries were removed for histologic processing and microscopic examination. In each case, the contralateral carotid artery was also removed for purposes of control and comparison. Samples of the injured carotid artery were taken both proximal and distal to the point of occlusion, and multiple sections were prepared from each of these samples.

The arterial segments were fixed in 10 percent neutral formalin (Lillie) for histologic study. The tissues were embedded in paraffin and sectioned at 3 μ. Frozen sections were cut at 5 to 10 μ. Adjacent sections were stained with hematoxylin and eosin for routine analysis, Alcian blue and toluidine blue for metachromasia, the Hale stain for mucopolysaccharides, Verhoeff van Gieson stain for elastic tissue, the von Kossa method for calcium, and Oil Red O and Sudan Black B for demonstration of lipids in the frozen sections.

**Results**

In general, there was no evidence of any inflammation at the site of ligation. The histopathologic reaction to injury was the same on both sides of the ligature extending proximally or distally for several centimeters. The pattern of histopathologic response to a standard arterial injury varied according to strain, i.e., S-D vs SHR, and the presence or absence of pre-existent arteriosclerosis, i.e., virgins vs breeders.

### A. Sprague-Dawley

**Histopathologic Response to Carotid Artery Ligation Injury. Normotensive, Nonarteriosclerotic Virgin Rats.** There were no discernible morphologic differences between male and female S-D rats in their reactivity to carotid artery injury. However, there was a dichotomous response between non-arteriosclerotic virgin and arteriosclerotic breeder rats (*vide infra*). None of the virgin rats had pre-existent arteriosclerosis, their blood pressure was normal, and there was active, concentric intimal proliferation leading to partial stenosis (± 50%) but not complete occlusion of the injured carotid artery (fig. 1, table). There was no evidence of medial involvement but the intima became markedly swollen with a pultaceous, mucopolysaccharide-rich ground substance, highly populated by mesenchymal or smooth muscle cells growing in a centripetal direction. These mesenchymal cells appeared to be orientated in an inner circular, outer longitudinal manner (fig. 1). This active myointimal proliferation advanced to complete occlusion in only a few rats and there was no evidence of thrombotic involvement (table).

**Moderately Hypertensive Breeder Rats with Pre-existent Arteriosclerosis.** Breeder rats with pre-existent arteriosclerosis and moderate hypertension, ranging from 136 to 155 mm Hg, manifested a different pattern of injury response characterized by complete occlusion of the lumen by extensive myointimal cellular ingrowth, thrombosis, or a combination of both (figs. 2, 3, table). The carotid arteries (intact and ligated) of the repeatedly-bred S-D rats were ectatic, associated with distention or thinning of the media. Myointimal proliferation was more intense in male breeders which characteristically develop less generalized arteriosclerosis than female breeders. Because of combined intimal ingrowth and thrombosis (fig. 3), occlusion was much more prevalent in female breeders with the more severe generalized arteriosclerosis (table). In several rats, protuberant, saccular aneurysms appeared at the site of ligation injury. The vectors of intimal proliferation and thrombus formation caused herniation of the carotid artery wall at foci of pre-existent medial elastolytic deterioration. These lesions...
TABLE: Summary of the Histopathologic Changes in the Carotid Arteries 3 Weeks Following Injury by Carotid Artery Ligation in Sprague-Dawley and Spontaneously Hypertensive Rats with and without Pre-existent Arteriosclerosis and Varying Degrees of Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Blood pressure (mm Hg)</th>
<th>Occclusion by cellular ingrowth</th>
<th>Total occlusion by thrombus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Partial (%)</td>
<td>Complete (%)</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virgin males</td>
<td>118 ± 4</td>
<td>0†</td>
<td>92</td>
</tr>
<tr>
<td>Virgin females</td>
<td>112 ± 5</td>
<td>0†</td>
<td>91</td>
</tr>
<tr>
<td>Breeder males-microscopic aortic lesions</td>
<td>147 ± 8</td>
<td>78†</td>
<td>9</td>
</tr>
<tr>
<td>Breeder females-early, grossly visible aortic lesions</td>
<td>142 ± 6</td>
<td>82†</td>
<td>8</td>
</tr>
<tr>
<td>Breeder females-advanced, grossly visible aortic lesions</td>
<td>145 ± 5</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virgin males</td>
<td>196 ± 4</td>
<td>0†</td>
<td>23</td>
</tr>
<tr>
<td>Virgin females</td>
<td>187 ± 5</td>
<td>0†</td>
<td>21</td>
</tr>
<tr>
<td>Breeder males-hyalinosis of testicular arterioles</td>
<td>221 ± 8</td>
<td>0†</td>
<td>0</td>
</tr>
<tr>
<td>Breeder females-hyalinosis of ovarian arterioles</td>
<td>208 ± 7</td>
<td>0†</td>
<td>0</td>
</tr>
</tbody>
</table>

*Twenty-four samples per group, blood pressure cited as Mean ± Standard Error, % indicates the approximate incidence of histopathologic changes, i.e., data compiled by analysis of alternate histological sections (4 samples of each carotid artery segment) rather than serial sections.

†Confirmed by microscopic examination.

*Remaining animals, i.e., 18%, were found to have microscopic aortic sclerosis.

stained more positively for mucopolysaccharide than those observed in non-arteriosclerotic virgin rats and some of these lesions stained positively for calcium but very little lipid.

B. Spontaneously Hypertensive Rats

Histopathologic Response to Carotid Artery Ligation Injury, Severely Hypertensive, Non-arteriosclerotic Virgin Rats. The virgin SHR were truly hypertensive with blood pressures ranging from 182 to 200 mm Hg (table). Despite their high blood pressure, there was no evidence of arterial disease, e.g., polyarteritis nodosa (PAN), etc. The pattern of response to arterial injury
CAROTID ARTERY INJURY IN SHR/Wexler

**Figure 4.** Carotid artery of a severely hypertensive male, virgin, SH rat free of arterial disease. The reaction to ligation injury is similar to that seen in normotensive S-D rats (cf. fig. 1) except that the arterial wall is thicker, the elastica of the media is thicker and more irregular than in S-D rats, and the proliferating intimal tissue stains less positively for mucopolysaccharide and is much more fibrotic. Verhoeff van Gieson, × 75.

was similar in non-arteriosclerotic but hypertensive virgin SHR vs the non-arteriosclerotic and normotensive S-D rats, i.e., myointimal or mesenchymal cell proliferation (fig. 4). However, there was a much higher incidence of occlusion because of greater cellular ingrowth in SHR vs S-D (table). The pultaceous intimal cushions of SHR stained much less positively for mucopolysaccharide and their elastic lamellae appeared to be thicker, more irregular, with extra prominent inter-lammelar bridges than is observed in most strains of rat (fig. 4).

**Severely Hypertensive Breeder Rats with Hyalinosis of the Testicular or Ovarian Arterioles.** Breeder SHR had slightly higher blood pressure levels than their virgin counterparts ranging from 201 to 229 mm Hg (table). There was a definite difference in their response to arterial injury compared to all other animals, i.e., total occlusion by thrombus formation (fig. 5). Of particular import was the appearance of prominent lipid-rich clefts, i.e., cholesterol complexes, interspersed throughout the thrombus. As with breeder S-D rats, the carotid arteries of breeder SHR were ectatic with

**Figure 5.** Carotid artery of a severely hypertensive, female, breeder SH rat having arteriosclerosis of the ovarian arteries but no aortic sclerosis prior to carotid artery ligation. The injured carotid artery wall is ectatic, the media is thinned, and the entire lumen is occluded by a thrombus containing numerous cholesterol crystals. H & E, × 75.
thinned but lesion-free media and no evidence of aneurysm formation as observed for breeder S-D with pre-existent arteriosclerosis.

Discussion

One of the most provocative aspects of this investigation is that normal, moderate, and severely elevated systolic blood pressure, as well as changes in the hormonal-metabolic milieu, i.e., due to repeated breeding, appeared to affect the morphologic response of the arterial wall in a diverse manner following standard injury. For example, although normotensive and non-arteriosclerotic S-D virgin rats respond by myointimal cellular ingrowth, the severely hypertensive and non-arteriosclerotic SHR virgin rats manifested much more intense myointimal hyperplasia and consequently a higher incidence of total occlusion of the lumen. This would suggest that the condition of elevated systolic blood pressure caused more intense myointimal proliferation. Further, it appears that the combination of moderately elevated blood pressure, pre-existent aortic sclerosis, and an altered hormonal-metabolic milieu, as found in breeder S-D, caused a shift in the response to injury favoring the appearance of thrombi concomitant with myointimal hyperplasia. Particularly striking is the appearance of an unusually high incidence of total occlusion by thrombosis only of the ligated carotid arteries of severely hypertensive breeder SHR. This is unusual because rats are resistant to thrombus formation. Since the virgin and breeder SHR had equally severe hypertension, it would appear that the altered hormonal-metabolic milieu in the breeder SHR, rather than the severe hypertension, was the key mechanism in conditioning their arteries toward such an unusual reaction, i.e., total occlusion by thrombosis only.

Several years ago, Buck described the growth of medial smooth muscle cells through the elastica interna in occluded carotid arteries making their way into the tunica intima and thence into circumferential proliferation. He called these cells "myointimal" cells. If platelets became attached, the myointimal cells and platelets would become organized as a complex thrombus. The intimal hyperplasia, arranged in an inner circular and outer longitudinal arrangement, which Buck observed is identical to what we have observed in this experiment and in ancillary experiments. Geer et al., Haust et al., and others have shown that smooth muscle cells are capable of synthesizing lipid, avascular organization, imbibing protein, fibrin, and fibrinogen, and being transformed into fibroblasts as they make their way into the intima. In this connection, it is of interest that the pultaceous intimal tissue in S-D rats stained very positively for mucopolysaccharides, whereas the more injury-reactive intimal tissue of SH rats was much less positive for mucopolysaccharide but stained very positively for collagen. Histologically, this would suggest that the mucopolysaccharide in the SHR lesions served as precursor material for collagen formation and that the presence of elevated blood pressure in the SH rats conditioned these mesenchymal or medial smooth muscle cells to differentiate into fibroblasts and extra collagen formation, i.e., the morphology and functional activity of medial smooth muscle cells depends on the physiochemical milieu.

The total absence of thrombus formation is normotensive, nonarteriosclerotic, virgin S-D rats vs extensive thrombotic involvement in mildly hypertensive and arteriosclerotic breeder S-D rats suggests that there is no intimal fibrin exposed in the normotensive, nonarteriosclerotic, virgin rats whereas the marked accumulation of intimal ground substance and collagen which occurs in hypertensive and arteriosclerotic breeder rats serves as an attractant and nidus for platelet clumping and thrombus formation. It is not clear why the severely hypertensive SH breeder rats responded to the injury by such extensive thrombus formation (leading to occlusion) since breeder SHR manifest hyalinosis of the arterioles of the gonads only and are free of aortic or carotid artery lesions. Clinically, it has been found that if the carotid artery is subjected to partial occlusion by clamping or arteriotomy, the media of the carotid artery becomes stretched and thin, as it did in our animals. This medial thinning is believed to be due to increased kinetic energy or to a decrease in the blood supply to the wall of the occluded artery. Injury of the carotid artery, either by clamping or by the manipulation of arteriotomy, is not attended by thrombosis. However, when the 2 kinds of trauma are combined, thrombosis becomes frequent. Thus, the absence of large occluding thrombi in S-D rats and the unusually high incidence of occluding thrombi in severely hypertensive SHR indicates that the trauma of occlusion, intimal outgrowth, and mild hypertension was not sufficient to set the stage for thrombosis. However, the combination of trauma and severe hypertension, plus some unknown factor in breeder SHR, did provide the proper milieu for total occlusion by thrombosis.

It is of interest that the clefts observed in the large occlusive thrombi found in breeder SHR carotid arteries stained intensely positive for lipid, i.e., cholesterol. Although repeatedly-bred rats of normotensive strains become obese, develop a fatty liver, and are hyperlipemic, even their most advanced arterial lesions contain very little lipid. In our strain of SHR, both virgin and breeder rats manifest spontaneous hyperlipidemia, yet their arterial lesions also contain little or no lipid. We believe that the cholesterol clefts observed within the thrombi were present by physical entrapment rather than by insudation.

Epidemiological surveys have amply demonstrated that hypertension promotes thrombosis and begets atherosclerosis and vice versa. Within the context of the experimental models described herein, the identification of severity of hypertension and specific hormones or metabolic vectors offers much promise in pinpointing those factors which condition the arterial wall toward degenerative changes.
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References

Histopathological reactivity of carotid arteries of normotensive Sprague-Dawley vs spontaneously hypertensive rats to ligation injury.

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