Morphologic and Functional Changes of the Aortic Intima During Experimental Hypertension

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The morphology and permeability to horseradish peroxidase of the rat aortic intima have been investigated in three experimental models of hypertension having different values of plasma renin content and plasma aldosterone level. During hypertension the aortic endothelium shows three main changes: 1) increased arithmetic mean thickness, with prominent rough endoplasmic reticulum and polyribosomes; 2) the appearance of actin microfilament bundles; and 3) increased permeability to horseradish peroxidase. These changes are not present in all models, do not appear to depend on hypertension per se, and are independent of each other. The subendothelial layer of hypertensive animals shows an increased thickness that appears to be correlated with an increase of endothelial cell volume. Our results suggest that: 1) the aortic intima reacts differently to different types of hypertension, and 2) factors other than hypertension per se play a role in the development of vascular changes observed in animals with elevated blood pressure. (Am J Pathol 96:399–422, 1979)

HYPERTENSION is one of the most important, if not the most important, predisposing factor for atheromatous lesions.[1] At present it is not established whether hypertensions with different pathogenetic mechanisms predispose equally to atheroma formation.[2,3] One of the typical changes of hypertensive disease is the thickening of the intima of large arteries.[4] It is conceivable that intimal changes alter the barrier function of this layer and thus promote the onset of atheromatous plaques. In this study, we have compared changes in the morphology and permeability of the aortic intima during early and late phases of three experimental models of hypertension. Our results indicate that the aortic intima reacts differently to different types of hypertension.

Materials and Methods

Experimental Models of Hypertension

Aortic Ligature Between Renal Arteries

One hundred thirty male Wistar rats weighing 200–300 g were used for this experiment. One group served as untreated controls (36 rats). In the second group, the aorta was

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ligated between the two renal arteries below the superior mesenteric artery.[5] Blood pressure was recorded 7 and 40 days after ligature of the aorta, with the animals under ether anesthesia, through the right carotid artery, with the use of a Narco Physiograph (Narco Bio-Systems, Inc., Houston, Tex) or a Hewlett-Packard 7702 B recorder (Hewlett-Packard, Geneva, Switzerland). Animals with more than 150 mm Hg systolic blood pressure were used in this group (32 rats 7 days and 24 rats 40 days after aortic ligature).

Unilateral Nephrectomy and an NaCl-Rich Diet

Ninety male Wistar rats weighing 150–160 g were divided into two groups. One group (30 rats) served as untreated control. In the second group (60 rats) the left kidney was removed with the animals under ether anesthesia, and the animals were given a Chow diet similar to that of the control group (U.A.R., Villemoisson sur Orge, France) but containing 6% NaCl. Blood pressure was recorded in control and treated animals at 7 and 40 days after the operation.

Unilateral Nephrectomy, 0.9% NaCl Solution as Drinking Fluid, and Subcutaneous Administration of Desoxycorticosterone Acetate (DOCA)

A group of 100 female Wistar rats weighing 150–160 g was used. Twenty rats kept on a normal diet and tap water served as controls: 80 rats underwent unilateral nephrectomy on the first day of the experiment and divided into four equal groups receiving respectively the following treatment: 1) none; 2) DOCA (11-desoxycorticosterone acetate, Serva Feinbiokemika, Heidelberg, West Germany) injected daily subcutaneously at a dose of 3 mg in 0.2 ml of distilled water (in a microcrystal suspension prepared by adding 1 drop of Tween-80 to every 10 ml of the solution); 3) a 0.9%-NaCl solution as drinking fluid; and 4) both DOCA and 0.9% NaCl drinking fluid. Blood pressure was recorded at 10 and 40 days after the beginning of the experiments.

Studies of Morphology and Permeability

During the continuous monitoring of blood pressure, horseradish peroxidase (HRP, Sigma Type II) was injected intravenously into rats of each experimental group in a dose of 5 mg/100 g body weight dissolved in 0.5 ml NaCl; this dose was chosen, after several trials, because it never caused any recognizable HRP reaction in the subendothelium of control animals by the time of fixation; hence we could always observe a qualitative difference (yes or no) between positive and negative animals. Five minutes after injection of HRP, perfusion with 2.5% glutaraldehyde in cacodylate buffer (pH 7.2) was carried out into the left ventricle of the heart via an 18-gauge needle connected to an infusion set. After perfusion for 10 minutes with about 80 ml of the fixative, the upper third of the thoracic aorta was isolated, cut into small blocks, and left for 5 hours in glutaraldehyde at room temperature. The blocks were washed overnight in cacodylate buffer at 4 C, and the HRP reaction product was developed as previously described; they were then postfixed in 2% OsO_4 in *s*-collidine buffer (pH 7.4), dehydrated in alcohol, and flat-embedded in Epon 812. In order to better delineate the endothelial cell membranes, some tissue cubes from each group were treated with uranyl acetate *en bloc* after postfixation.[6] Unstained sections, 1 μ in thickness, were prepared from all Epon-embedded aortic segments and were reviewed by light microscopy. From selected blocks, thin sections were cut on LKB III ultratomes (LKB-Produckter AB, Stockholm, Sweden) or Reichert ultramicrotomes (C. Reichert, AG, Vienna, Austria) and examined, either unstained or following lead citrate or combined uranyl acetate and lead citrate staining, with Philips EM-300 microscopes.

Morphometry

For morphometric examination, 70×70-mm or 35×35-mm pictures were taken randomly at the constant magnification of 3.670 for 70×70-mm and of 936 for 35×35-mm pictures (5 rats per group, 2 tissue blocks per rat, 8 pictures per block). Positive films of these images were projected on a standard morphometric screen containing 84 parallel

horizontal lines 2 cm in length. For endothelial and subendothelial layers, we calculated the arithmetic mean thickness, which can be defined as the average volume of tissue falling on the unit area of its surface. This corresponds to the general formula

$$\bar{\tau} = \frac{Z \times P_i}{2 \times I_i}$$

where P_i = the number of the end points of screen lines lying within the endothelium or subendothelium (corresponding to the volume of these structures); I_i = the number of intersections of the lines with the external or internal surfaces of endothelium and subendothelium (corresponding to the surface of these structures); and Z = the length of a line of the test grid divided by the magnification used.[7] The evaluation of the results was done by means of the Student *t* test.

Immunofluorescence

En face preparations of endothelial cells were made[8] from thoracic aortas of at least 4 rats per group. The preparations were treated either with normal human serum or with serum from a patient with chronic aggressive hepatitis containing antiactin antibodies (AAA),[9,10] and were then stained with fluorescein-conjugated IgG fraction of goat antiserum to human IgG (Code 64.170, Miles Seravac, Lausanne, Switzerland) according to a technique described previously.[5] The specificity of the AAA serum was assessed by immunodiffusion and immunoabsorption.[5,9,10]

Biochemical Determinations

The rats to be examined (42 control animals; 22 rats 7 days after ligature of the aorta; 9 rats 40 days after ligature; 4 rats 7 days after the sodium-rich diet; 8 rats 40 days after the soduim-rich diet; 9 rats 10 days after unilateral nephrectomy, 0.9% NaCl in drinking fluid, and DOCA; and 5 rats 40 days after unilateral nephrectomy, 0.9% NaCl in drinking fluid, and DOCA) were placed in metabolic cages for 3 days. Their body weight, water intake, and urine excretion was measured daily. Sodium excretion was determined by flame photometry. On the fourth day the animals were killed by decapitation,[11] and their blood was collected in tubes containing 14 mg EDTA (6 mg for 5 ml whole blood) at 0 C. Plasma was obtained by centrifugation of the blood at 50*g* for 15 minutes, and diisopropyl fluorophosphate was added as enzymatic inhibitor. The plasma renin content (PRC) was assessed in the following way: 50 μl plasma per rat was adjusted to the optimum of pH of 6.5; 50 μl (40 mg) of rat renin substrate, purified according to the method of Skeggs,[12,13] and 10 μl diluted angiotensin I antiserum was added; the samples were incubated 1 hour at 37 C with continuous agitation. The angiotensin I generated was directly related to the renin content (since excess substrate had been added to reach zero-order kinetic) and was measured by the radioimmunoassay technique previously described[14] and modified to include the trapping antibody.[15] The angiotensin I antiserum used had been raised in Dr. Vallotton's laboratory, and its specificity thoroughly tested.[16,17] Plasma from at least 3 rats was pooled for plasma aldosterone level (PAL) evaluation by radioimmunoassay with the use of antiserum A3-35 from Endocrine Science (Tarzana, Calif). In DOCA-treated animals, prior to essay of aldosterone in methylene chloride extract of plasma, DOCA was preextracted with CCl_4.

Results

Aorta Ligature Between Renal Arteries

The systolic and diastolic blood pressures of untreated rats at 7 days (20 rats) and at 40 days (16 rats) were 105/80 mm Hg (SEM = 4/6) and

117/86 mm Hg (SEM = 3/4), respectively. In rats with aortic ligature, the blood pressure above the ligature averaged 179/142 mm Hg (SEM = 6/6) in the 7-day group (32 rats) and 192/135 mm Hg (SEM = 7/5) in the 40-day group (24 rats).

In thick sections, the endothelium of control thoracic aortas was identified as a flat cell layer lying close to the internal elastic lamina (Figure 1A). The subendothelial layer was devoid of HRP reaction product following 5 minutes tracer circulation time. As compared with controls, the aortic segments 7 days after the operation had a thickened endothelial cell layer, and HRP reaction product was found to be very intense in the subendothelial space (Figure 1B). Forty days after the operation, the aortic endothelium formed an undulated layer lying above the subendothelium; however, the size of endothelial cells in most segments examined was comparable to that of control aortas. The subendothelial layer, clearly visible between the endothelium and the internal elastic lamina, was devoid of HRP reaction in most aortic segments examined (Figure 1C).

On electron-microscopic examination, the aortic intima of normotensive controls (Figure 4) was as previously described.[18-20] In particular, adjacent to interendothelial spaces, few cytoplasmic microfilaments were noted; such filaments have been more commonly demonstrated in arteriolar endothelium.[21-24] Endothelial cells were interconnected by both tight and gap junctions.[18] Unstained sections revealed HRP reaction product in occasional plasmalemmal vesicles and multivesicular bodies; the subendothelial layer, however, was free of reaction product. One week after aortic ligature, endothelial cells of thoracic aortas from hypertensive rats bulged into the lumen and showed many microvillous and/or flap-like cytoplasmic projections. The endothelial cell cytoplasm exhibited prominent rough endoplasmic reticulum, free ribosomes, and Golgi complex. In addition, bundles of microfilaments were present in the cytoplasm (Figure 5), being mainly localized at the periphery of the cell.[5,25-30] HRP reaction product was localized in plasmalemmal vesicles of endothelial cells, within some interendothelial clefts, and was uniformly distributed throughout the subendothelial layer (Figure 6), which appeared thickened (Figures 5 and 6). Occasional macrophages and/or lymphocytes were noted in the subendothelial space. At 40 days, aortic endothelial cells had irregular contours but were smaller than those of the 7-day group (Figure 7). Cytoplasmic microfilaments had practically disappeared by this time, when compared to endothelial cells of animals at 1 week after ligature. The endothelial cell layer was separated from the internal elastic lamina by an acellular connective tissue matrix consisting of basement lamina-like material associated with collagen and elastic fibers. HRP

reaction product was present in occasional endothelial vesicles. A few macrophages located in the subendothelial layer contained HRP in dense bodies and vesicles, but HRP reaction product was not seen in the subendothelial connective tissue compartment.

The results of morphometric evaluation of endothelial and subendothelial layer thickness are shown in Table 1. Evaluation of these results by means of a Student t test showed for the endothelium a significant difference between normotensive and hypertensive rats 7 days after ligature of the aortas ($2P < 0.001$) and between hypertensive animals 7 and 40 days after ligature ($2P < 0.001$). There was no significant difference between normotensive animals and animals 40 days after ligature of the aorta ($2P > 0.3$). For the subendothelial layer, the evaluation of the results showed a significant difference between normotensive rats and rats 7 days after ligature of the aorta ($2P < 0.001$) and between normotensive animals and animals 40 days after ligature of the aorta ($2P < 0.001$). No significant difference was present between hypertensive animals 7 and 40 days after ligature of the aorta ($2P > 0.3$).

No fluorescence was seen in any *en face* preparations after treatment with normal serum (Figure 8A). After incubation with AAA serum, fluorescence was only barely visible in the cytoplasm of the endothelial cells of control animals (Figure 8B). Intense fluorescence was seen in the cytoplasm of the majority of endothelial cells prepared from thoracic aortas 7 days after operation (Figure 8C). In endothelial cells of the 40-day group,

Table 1—Morphometric Evaluation of Aortic Intima Thickness During Experimental Hypertension

Treatment	Mean thickness (μ ¹)	
	Endothelium	Subendothelium
None*	1.1492 ± 0.1042	0.3071 ± 0.0297
Aortic ligature 7 days	3.1411 ± 0.3264	2.1052 ± 0.3668
Aortic ligature 40 days	0.9910 ± 0.1321	2.6543 ± 0.3954
None*	0.7988 ± 0.0504	0.3767 ± 0.0337
Uninephrectomy + Na-rich diet 7 days	0.9785 ± 0.0694	0.4247 ± 0.0684
Uninephrectomy + Na-rich diet 40 days	0.9997 ± 0.0963	2.1619 ± 0.2163
None*	0.7844 ± 0.0357	0.2963 ± 0.0144
Uninephrectomy + 0.9% NaCl + DOCA 10 days	1.5420 ± 0.1269	1.1120 ± 0.0956
Uninephrectomy + 0.9% NaCl + DOCA 40 days	1.1001 ± 0.0504	3.2983 ± 0.3696

* For the sake of clarity, only one group of controls is presented for each experiment, since there were no statistically significant differences between the controls of early and late groups.

fluorescence was much less intense than at 7 days (Figure 8D). In preparations treated with AAA serum previously incubated with skeletal muscle actin,[10] no fluorescence was present.

PRC and PAL values are shown in Table 2. In both cases, the values were increased in animals 7 days after ligature of the aorta ($2P < 0.025$ for PRC, whereas for PAL the statistical evaluation was not possible because of pooling of the sera); the values were not changed in animals 40 days after ligature, compared to those of normotensive animals.

Unilateral Nephrectomy and the Na-Rich Diet

Systolic and diastolic blood pressure of control rats at 7 and 40 days was 113/80 mm Hg (90/70–120/90) and 125/95 mm Hg (105/80–130/100), respectively.

In rats that had undergone unilateral nephrectomy and were on the high-salt diet, blood pressure was 120/80 mm Hg (105/70–130/85) in the 7-day group and 186/124 mm Hg (160/110–210/140) in the 40-day group.

In thick sections, the endothelium of control thoracic aortas was identified as a flat cell layer applied closely to the internal elastic lamina (Figure 2A). The subendothelial layer was devoid of HRP reaction product after 5

Table 2—Plasma Renin Concentration and Plasma Aldosterone Level During Different Types of Experimental Hypertension

Treatment	Plasma renin concentration (mean ± SEM) (ng•ml^{-1}•h^{-1})		Plasma aldosterone levels (mean of pools) (ng•dl^{-1})	
	Control	Experimental	Control	Experimental
Aorta ligature				
7 days	10 ± 1 (18)*	32 ± 4 (22)	7 (6) (3.9–11)†	97 (6) (25–215)
40 days	8.6 ± 2.7 (4)	8.9 ± 1.7 (9)	3.9 (2) (3.3–4.6)	5.5 (4) (3.5–8.5)
Uninephrectomy + Na-rich diet				
7 days	8.9 (2)	1.2 ± 0.2 (4)	4.7 (1)	2 (1)
40 days	7.4 ± 0.8 (10)	2.8 ± 0.6 (8)	2.9 (2) (1.7–4.2)	<1 (2)
Uninephrectomy + 0.9% NaCl + DOCA				
10 days	10 ± 1 (5)	0.8 ± 0.1 (9)	6.5 (3) (4.7–8.6)	2.3 (3) (1.5–3.5)
40 days	6.6 ± 0.1 (3)	0.8 ± 0.3 (5)	8.4 (1)	6.6 (3) (4.4–11.1)

* Number of determinations
† Range

minutes tracer circulation time. Compared to normotensive controls, no difference was visible in the group of animals 7 days after the Na-rich diet (Figure 2B). At 40 days, the size of endothelial cells in most segments examined was comparable to that of control aortas (Figure 2C). The subendothelial layer was clearly visible between the endothelium and the internal elastic lamina. HRP reaction product was not detected in the subendothelial layer in either experimental group.

On electron-microscopic examination, the aortic intima of normotensive rats was as previously described (Figure 4).[18-20] Unstained sections revealed HRP reaction product in occasional plasmalemmal vesicles. The subendothelial space, however, was free of tracer. At 7 days after unilateral nephrectomy and the Na-rich diet, aortic endothelial cells and subendothelium were similar to those of normotensive control animals. After 40 days, the fine structure of endothelial cells was again similar to that of normotensive animals (Figure 9). However, the subendothelium by this time contained an organized connective tissue matrix with the texture of a complex network of multilayered reticular basal laminas (Figure 9). HRP reaction product was not found in the subendothelial space of any aortic segments examined.

The results of morphometric evaluation of endothelial and subendothelial layer thickness are shown in Table 1. Evaluation of these results by means of the Student t test showed for the endothelium a slight but significant difference between normotensive control animals and animals at 7 ($2P < 0.05$) and 40 ($2P < 0.05$) days after the Na-rich diet. For the subendothelial layer, the evaluation of the results showed no significant difference ($2P > 0.4$) between normotensive rats and rats 7 days after the Na-rich diet; a significant difference was present between normotensive rats and rats 40 days after the Na-rich diet ($2P < 0.001$) and between rats 7 and 40 days after the Na-rich diet ($2P < 0.001$).

No fluorescence was seen in any *en face* preparations after treatment with normal serum. After incubation with AAA serum, only a weak fluorescence was detected at the periphery of the endothelial cells both in controls and in treated animals, indicating that there was no difference in cytoplasmic contractile protein content.

PRC and PAL values are shown in Table 2. In both cases, the values were decreased 7 and 40 days after the Na-rich diet when compared to controls (for PRC, $2P < 0.025$ at 7 and 40 days, whereas for PAL the statistical evaluation was not possible because of pooling of the serums).

Unilateral Nephrectomy, 0.9% NaCl in Drinking Fluid, and Subcutaneous Administration of DOCA

In untreated controls, blood pressure was 115/90 mm Hg (110/80–125/95) at 10 days and 120/80 (105/65–125/95) at 40 days. In uni-

nephrectomized rats, blood pressure was 113/86 (105/75–125/95) at 10 days and 120/90 (100/70–130/100) at 40 days. In uninephrectomized rats receiving DOCA, blood pressure was 140/85 (125/80–150/95) at 10 days and 150/107 (135/100–165/115) at 40 days. In uninephrectomized rats receiving 0.9% NaCl in drinking fluid, blood pressure was 115/80 (105/75–125/90) after 10 days and 125/90 (110/80–130/95) after 40 days, and in uninephrectomized rats receiving 0.9% NaCl in drinking fluid and DOCA subcutaneously blood pressure was 161/112 (150/165–175/135) at 10 days and 177/110 (150/80–200/120) after 40 days.

In thick sections the endothelium of control thoracic aortas was as described previously, and the subendothelial layer was devoid of HRP reaction product after 5 minutes tracer circulation time (Figure 3A). The aortic segments of animals 10 days after unilateral nephrectomy or unilateral nephrectomy and 0.9% NaCl in drinking fluid were similar to those of controls. The aortic segments of animals 10 days after unilateral nephrectomy and DOCA or after unilateral nephrectomy, 0.9% NaCl, and DOCA had a thickened endothelial layer (more evident in the second group); and HRP reaction product was found in the subendothelial space, focally in the group receiving only DOCA and constantly in the group receiving 0.9% NaCl and DOCA (Figure 3B). After 40 days the findings were similar to those seen after 10 days. However, the endothelial layer of uninephrectomized animals receiving DOCA alone or 0.9% NaCl and DOCA formed an undulated layer (less prominent than after 10 days) above the subendothelium (Figure 3C).

In untreated animals, the ultrastructure of the aortic intima was as previously described.[18-20] In particular, no HRP reaction product was present in the subendothelial space after 5 minutes circulation time. In uninephrectomized animals and in uninephrectomized animals receiving 0.9% NaCl in drinking fluid, the ultrastructure of aortic intima was similar to that of controls, and no HRP reaction product was present in the subendothelium. In the groups of uninephrectomized rats receiving DOCA and DOCA plus 0.9% NaCl in drinking fluid, the changes of the aortic intima were qualitatively similar and will therefore be described together. The only difference between these two groups was that in uninephrectomized animals receiving NaCl plus DOCA the changes were generalized, whereas in uninephrectomized animals receiving only DOCA the changes were focal and not as marked as in the other group. Ten days after treatment, aortic endothelial cells were enlarged, with an increased number of cytoplasmic organelles, such as dilated rough endoplasmic reticulum, polyribosomes, microtubules, mitochondria, and Golgi complexes (Figure 10). Many endothelial cells contained bundles of

cytoplasmic microfilaments (40–70 Å in diameter with a few filaments 100–120 Å in diameter scattered in between) (Figure 11). The bundles were visible throughout the cytoplasm, but they were most frequently located at the cell periphery. HRP reaction product was visible in plasmalemmal vesicles of endothelial cells, within some interendothelial clefts, and in the subendothelial space. The subendothelium was enlarged when compared to that of control animals and contained basement-membrane–like material associated with some collagen and elastic fibers (Figure 10).

After 40 days, the endothelial and subendothelial layers of uninephrectomized animals and of uninephrectomized animals receiving the 0.9% NaCl solution were similar to those of normotensive control animals. No HRP reaction product was present in the subendothelium. In animals receiving DOCA and DOCA plus the 0.9% NaCl solution, the changes were again similar but less marked in the first group. Aortic endothelial cells had irregular contours and were smaller than those of the 10-day group. Cytoplasmic microfilaments were decreased, when compared to those present in endothelial cells of animals 10 days after treatment. Endothelial cells were separated from the internal elastic lamina by a thick layer of basement-lamina–like material associated with collagen and elastic fibers (Figure 12). Within this layer, several inflammatory cells, mostly lymphocytes and macrophages, were visible, together with cell debris, often in the form of large osmiophilic bodies. HRP reaction product was seen in some endothelial vesicles and within segments of interendothelial spaces and was constantly present in the subendothelial layer of animals receiving DOCA as well as in animals receiving DOCA plus NaCl.

The results of morphometric evaluation (which were limited to the uninephrectomized group receiving NaCl and DOCA, since no qualitative differences were seen between the group and the animals receiving only DOCA) of endothelial and subendothelial layer thickness are shown in Table 1. Evaluation of these results by means of the Student t test showed for the endothelium a significant difference between controls and animals 10 ($2P < 0.001$) and 40 ($2P < 0.005$) days after unilateral nephrectomy, NaCl, and DOCA as well as a significant ($2P < 0.005$) difference between the groups after 10 and 40 days. For the subendothelial layer, the evaluation of the results showed a significant difference between control rats and animals 10 ($2P < 0.001$) and 40 ($2P < 0.001$) days after uninephrectomy, NaCl, and DOCA, as well as a significant difference ($2P < 0.001$) between the two groups after 10 and 40 days.

PRC and PAL values are shown in Table 2. In both cases, the values

were decreased, compared to those of control animals, in animals 10 days after uninephrectomy, NaCl, and DOCA ($2P < 0.025$ for PRC, whereas for PAL the statistical evaluation was not possible because of pooling of the serums). In animals 40 days after uninephrectomy, NaCl, and DOCA, PRC was decreased ($2P < 0.025$) and PAL was unchanged, compared to that of control animals.

Discussion

The early events leading to atheroma formation remain mysterious despite intense studies carried out in many laboratories. Recently, emphasis has been placed on factors inducing smooth-muscle proliferation, such as mutagens [31] or mitogens,[32] which would initiate the pathogenetic chain ending with the typical atheroma. Probably, the endothelial and subendothelial barriers regulate the availability of these substances for smooth muscle; thus, the study of intimal changes during situations that predispose for atheroma formation (eg, hypertension) may help in understanding the pathogenetic mechanism of this lesion.

Our results (summarized in Table 3) show that during different types of hypertension, some changes of the aortic intima are common to all models, while others develop only in certain types of hypertension.

The aortic endothelium of hypertensive animals shows three main changes: 1) hypertrophy of endothelial cells (as quantified by the increase of the arithmetic mean thickness of the endothelial cell layer); 2) the appearance of cytoplasmic microfilament bundles; and 3) an increase of permeability to HRP.

Endothelial-cell hypertrophy is very mild throughout the experiment with the Na-rich diet. It is very marked in the early phases following either ligature of the aorta or uninephrectomy, NaCl, and DOCA; how-

Table 3—Morphologic and Biochemical Changes During Different Types of Hypertension

Treatment	Blood pressure	Endothelial layer thickness	Sub-endothelial layer thickness	Permeability to HRP	Plasma renin concentration	Plasma aldosterone level
Aorta ligature						
Early	179/142	↑	↑	↑	↑	↑
Late	192/135	Unchanged	↑	Unchanged	Unchanged	Unchanged
Na-rich diet*						
Early	120/80	↑	Unchanged	Unchanged	↓	↓
Late	186/124	↑	↑	Unchanged	↓	↓
0.9% NaCl + DOCA*						
Early	161/112	↑	↑	↑	↓	↓
Late	177/110	↑	↑	↑	↓	Unchanged

* Rats unilaterally nephrectomized

ever, in the first model it regresses completely by 40 days, despite the persistence of hypertension, while in the second model it regresses only partially. Hypertrophy of aortic endothelial cells with prominent rough endoplasmic reticulum and polyribosomes suggests an increased synthetic activity of these cells that correlates well with the deposits of basement-lamina–like material in the subendothelium.[33]

Our experiments do not allow a conclusion on the mechanism of aortic endothelial-cell hypertrophy. It has been recently shown that during acute hypertension produced by ligature of a renal artery in the rat, there is an increased replication rate of aortic endothelial cells when compared to normal animals.[34] Using the same technique,[34] we have found (unpublished experiments) an increased replication rate of endothelial cells 7 days after ligature of the aorta; whereas the replication rate of endothelial cells reverts to normal 40 days after ligature, when the size of endothelial cells is similar to that of control animals, thus suggesting that endothelial-cell hypertrophy and replication are adaptive responses to an increase of blood pressure.

Under our experimental conditions, actin microfilament bundles develop constantly at the early phase after aortic ligature and after uninephrectomy, NaCl, and DOCA, when the endothelial cells are significantly thickened. We do not know the function of these microfilament bundles. However, the presence of cytoplasmic contractile proteins in nonmuscle cells has been recently related to such phenomena as cell contraction,[35,36] cell movement,[37] exocytosis, and endocytosis.[38,39]

Increased permeability to HRP is present at the early phase after ligature of the aorta and at the early and late phases after uninephrectomy, 0.9% NaCl, and DOCA. The comparison between permeability changes and biochemical evaluations (Table 3) shows that there is a definite correlation between the increase of circulating endogenous or exogenous mineralocorticoids and the increase of aortic endothelial permeability to HRP. Increased permeability to HRP coincides with an important thickening of the subendothelial layer, such as those seen early after aortic ligature and after uninephrectomy, 0.9% NaCl, and DOCA or 40 days after uninephrectomy, 0.9% NaCl, and DOCA, when, contrary to aortic ligature, the subendothelial layer has further increased in thickness, compared to that in the early phase. This suggests that in addition to synthetic activity of endothelial cells, increased permeability of the endothelial layer to circulating substances plays a role in the production of subendothelial thickening.

The subendothelial layer of hypertensive animals was always thickened; however, the patterns of thickening were different in each model.

After ligature of the aorta, an important subendothelial layer was already visible after 7 days and did not progress significantly up to 40 days. In uninephrectomized animals receiving the Na-rich diet, no subendothelial thickening was present after 7 days (when blood pressure was normal), but it developed progressively up to 40 days. In uninephrectomized animals receiving 0.9% NaCl and DOCA, a significant thickening was present already after 7 days, and it progressed significantly up to 40 days. Little is known about formation and turnover of the subendothelial layer in large vessels. In normal rats, it has been proposed that the basement membrane of the aorta is synthetized during the early months of life by the endothelium, which at that time shows hypertrophy of rough endoplasmic reticulum.[40] In small vessels, the thickening of the basement membrane results probably from endothelial cell injury.[33] Our results indicate that there is a correlation between the development of subendothelial thickening and the thickness of the endothelial cell layer. Subendothelial thickening develops slowly in animals receiving the Na-rich diet, which show only a small degree of endothelial hypertrophy throughout the experiment; whereas at the early phase after aorta ligature or after uninephrectomy, 0.9% NaCl, and NOCA, subendothelial and endothelial thicknesses are concurrently increased. Subendothelial thickening does not progress significantly between 7 and 40 days after aortic ligature, when, despite the persistence of hypertension, endothelial cell volume decreases; whereas it progresses significantly after uninephrectomy, 0.9% NaCl, and DOCA, where endothelial cells remain hypertrophic throughout the experiment. We do not know the composition of the material deposited in the subendothelial layer during hypertension; however, our results support the possibility that an increased synthetic activity of endothelial cells is at least in part responsible for the increased deposition of basement-lamina–like material in the subendothelial layer.

PRC and PAL show important differences in our experimental models. Seven days after ligature of the aorta, PRC is increased in comparison with that of normal animals [41,42]; this situation in turn stimulates aldosterone secretion.[43] Forty days after ligature, both PRC and PAL are significantly decreased.[41,42,44] After uninephrectomy and the Na-rich diet,[45] or after uninephrectomy, 0.9% NaCl, and DOCA administration, both PRC and PAL are decreased or unchanged when compared to controls. However, in the later model, circulating exogenous DOCA is clearly elevated, and it is well established that the administration of DOCA results in low levels of plasma renin activity [46,47] such as those observed in our experiments. As we have seen above, there is a correlation between the increase

of circulating endogenous or exogenous mineralocorticoids and the increase of endothelial permeability to HRP.

We do not find any relationship between the increase of PRC and any of the morphologic or functional changes observed. It has been proposed that in humans not all types of hypertension predispose to the same extent for atheromatous changes, the incidence of cardiovascular complications being higher in hypertensive patients with high PRC than in those with low PRC.[2,3] However, the validity of the statistical evidence for this suggestion has been challenged,[48-51] and experimental data in this field are conflicting.[52,53] In experimental animals, it has been shown that the acute administration of angiotensin increases endothelial permeability in arteries and arterioles.[54-56] Our results do not support the correlation between increased levels of PRC and the presence of changes in morphology and permeability in the aortic intima. Indeed, hypertension produced by uninephrectomy, 0.9% NaCl, and DOCA results in a high degree of endothelial hypertrophy, subendothelial thickening, and permeability to HRP but is accompanied by low levels of PRC while obviously high amounts of DOCA are present in the animals.

In conclusion, our results show that the reaction of aortic intima during hypertension differs in the various experimental models studied, thus indicating that 1) the aortic intima reacts differently to different types of hypertension and/or it may develop an adaptive reaction to high blood pressure, and 2) factors other than hypertension per se play a role in the development of vascular changes observed in animals with elevated blood pressure.

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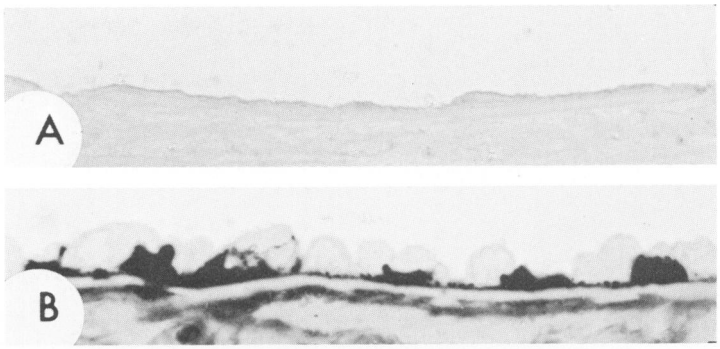

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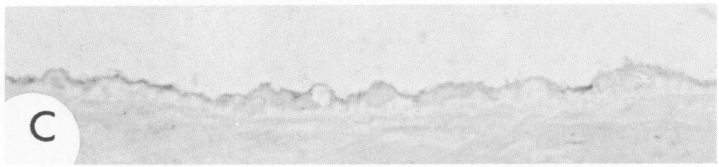

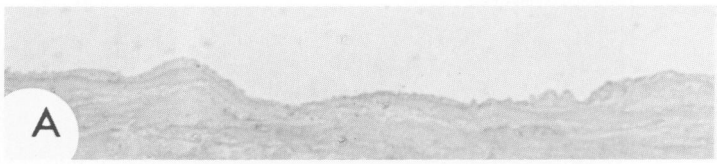

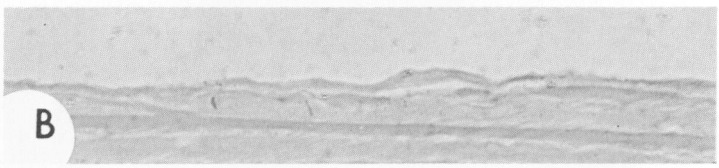

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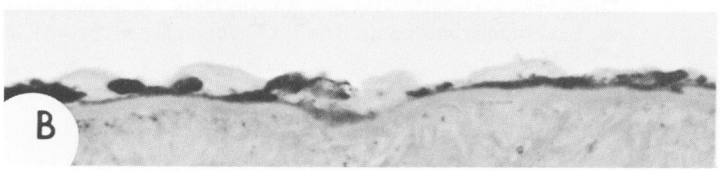

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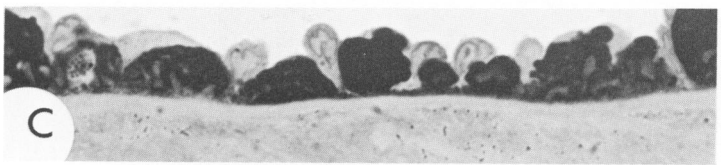


Figures 1-3—Comparative light micrograph of unstained thick sections of thoracic aortas 5 minutes after injection of HRP. **Figure 1 A**—Control rat. **Figure 1 B**—Hypertensive rat 7 days after aortic ligature. **Figure 1 C**—Hypertensive rat 40 days after aortic ligature. Note hypertrophy of endothelial cells and dark HRP reaction product in the subendothelium at 7 days after aortic ligature. At 40 days, in spite of high blood pressure, the subendothelium is negative for HRP reaction. **Figure 2 A**—Control rat. **Figure 2 B**—Rat 7 days after the Na-rich diet. **Figure 2 C**—Rat 40 days after Na-rich diet. The endothelial layer appears similar in the three groups. The subendothelial layer appears thickened only at 40 days, and HRP reaction product is never visible. **Figure 3 A**—Control rat. **Figure 3 B**—Rat 10 days after unilateral nephrectomy, 0.9% NaCl in drinking fluid, and DOCA. **Figure 3 C**—Rat 40 days after the same treatment. Note hypertrophy of endothelial cells and HRP reaction product in the subendothelium after 7 and 40 days. The subendothelium appears thicker after 40 days when compared to that of 7 days. (×1000)

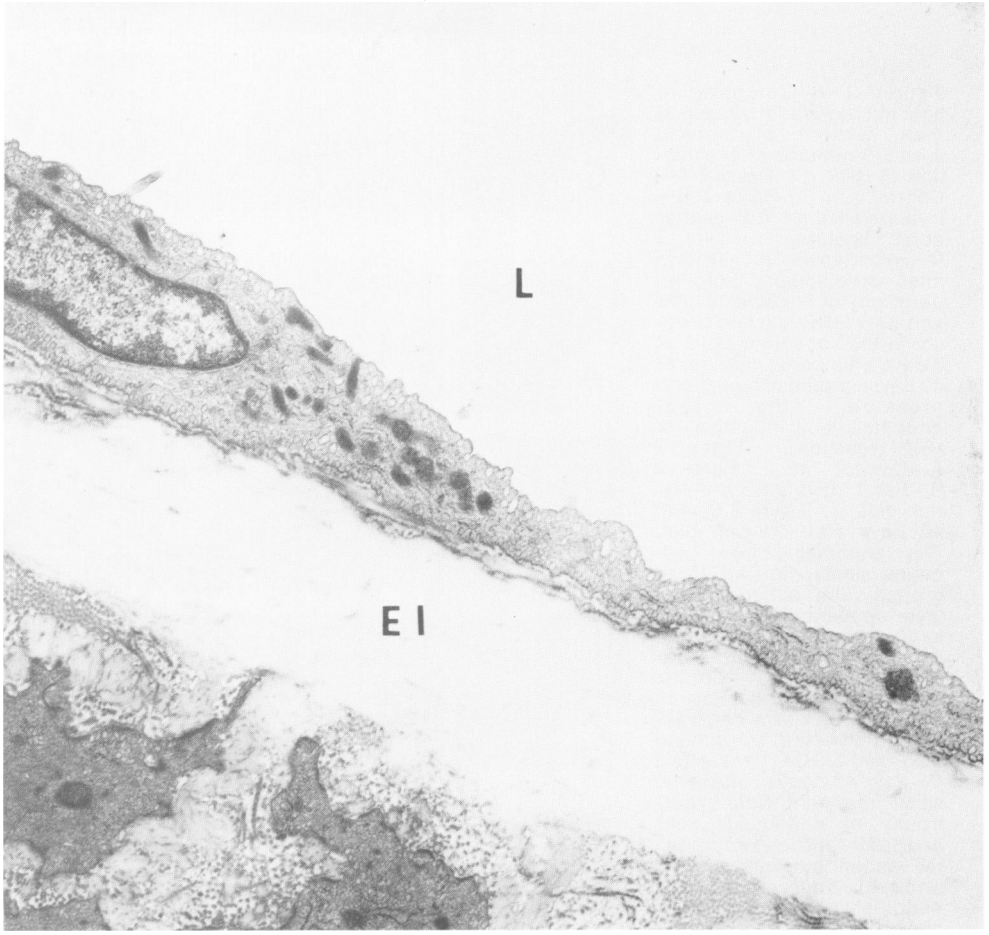


Figure 4—Intima of the thoracic aorta in a normotensive rat. The endothelial cell nucleus has a regular contour. The cytoplasm contains mitochondria, rough endoplasmic reticulum, and pinocytotic vesicles, but not microfilaments. The subendothelial layer is very thin. *L* = lumen; *EI* = elastica interna. (×13,000)

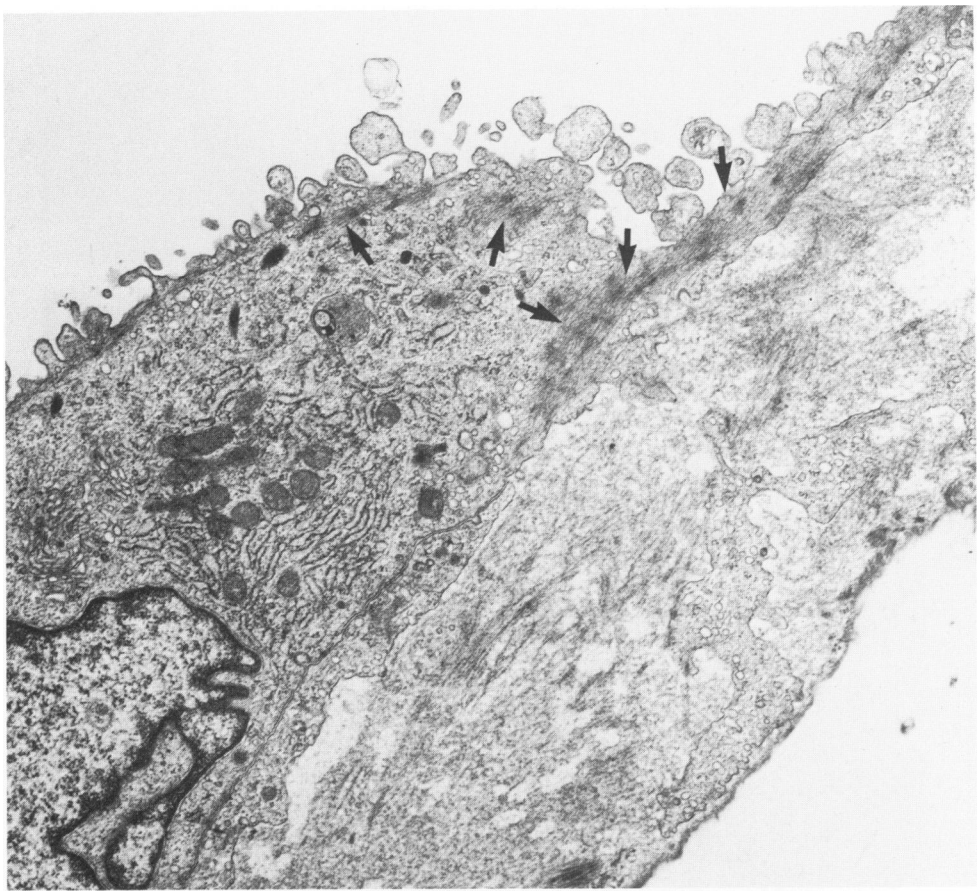
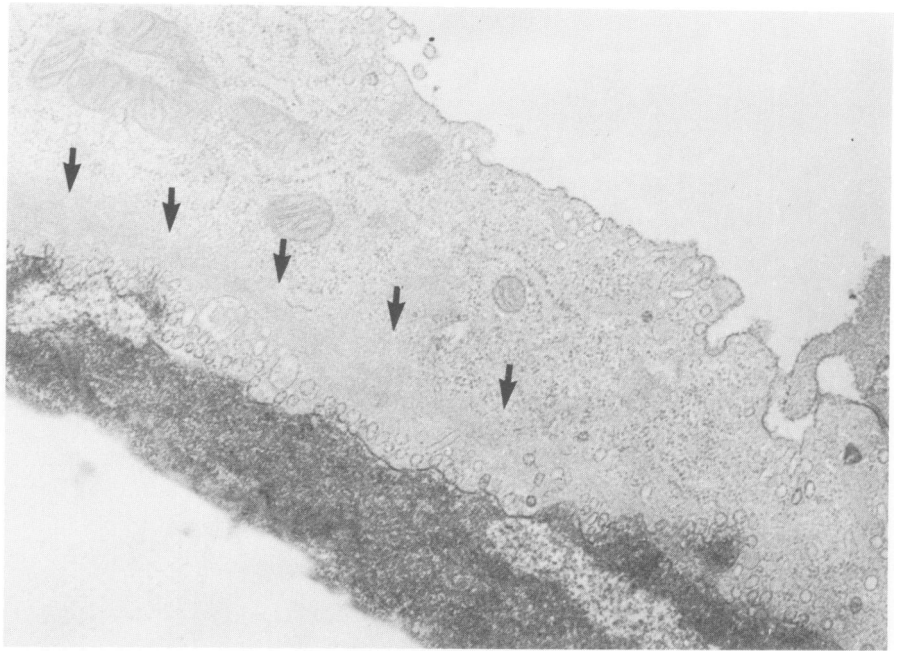


Figure 5—Intima of the thoracic aorta of a rat 7 days after ligature of the aorta between the renal arteries. The endothelial cell is hypertrophic. Note the bundle of cytoplasmic microfilaments (*arrows*). Dense areas similar to "attachment sites" are distributed throughout the microfilament bundles. The subendothelial layer is thickened and contains basement-lamina-like material. (×13,000)



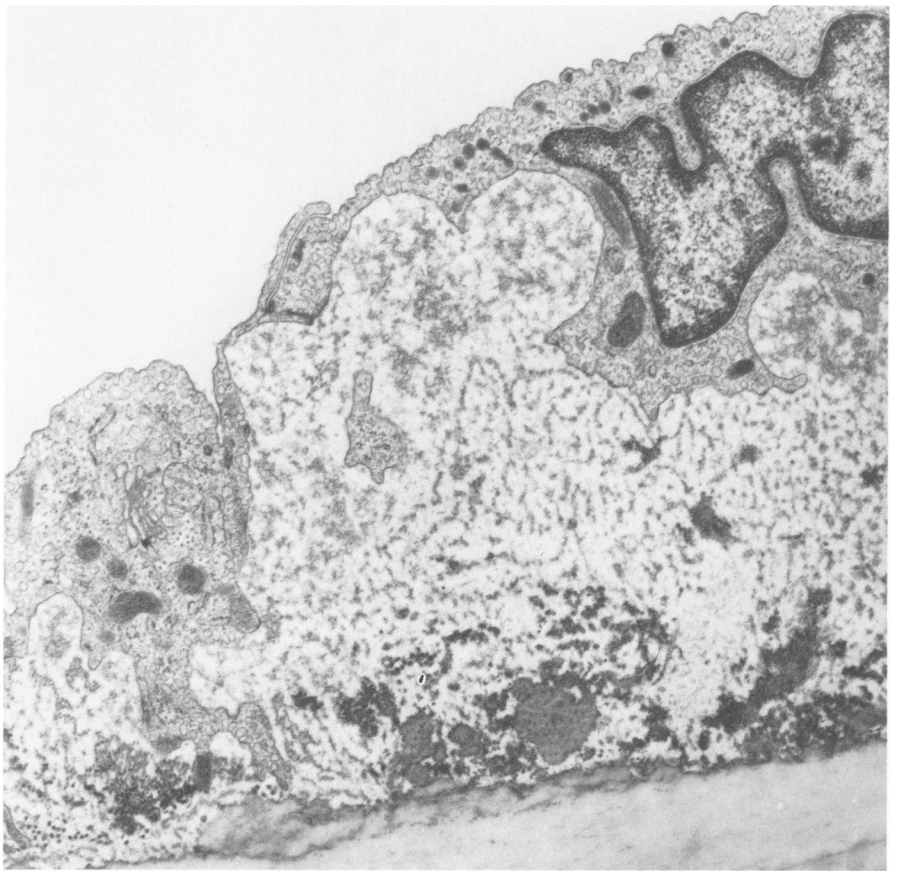
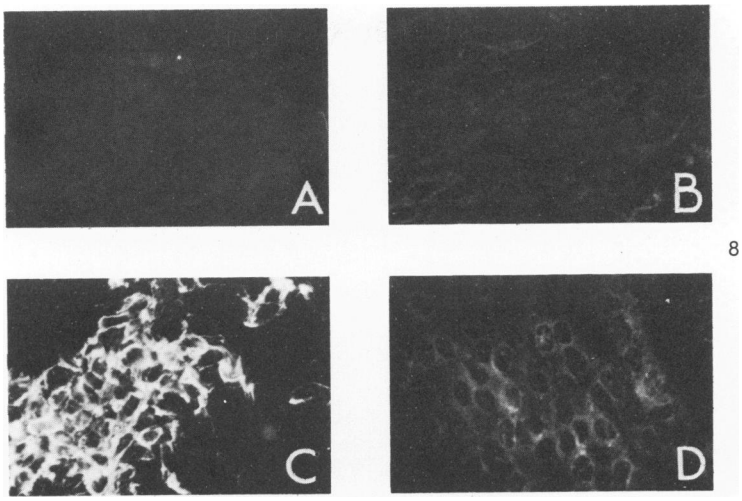


Figure 6—Unstained section of aortic endothelium of an hypertensive rat 7 days after ligature of the aorta between the renal arteries. The rat has received HRP intravenously for 5 minutes. The endothelial cell contains a bundle of microfilaments (*arrows*). The subendothelial space is filled with HRP reaction product, which is also present in a few pinocytotic vesicles of the endothelial cytoplasm. (×22,800). **Figure 7**—Intima of the thoracic aorta in a hypertensive rat 40 days after ligature of the aorta between the two renal arteries. The size of endothelial cells is similar to that of those of normotensive animals. However, the subendothelial layer remains thickened and contains large amounts of basement-lamina-like material. (×13,000)



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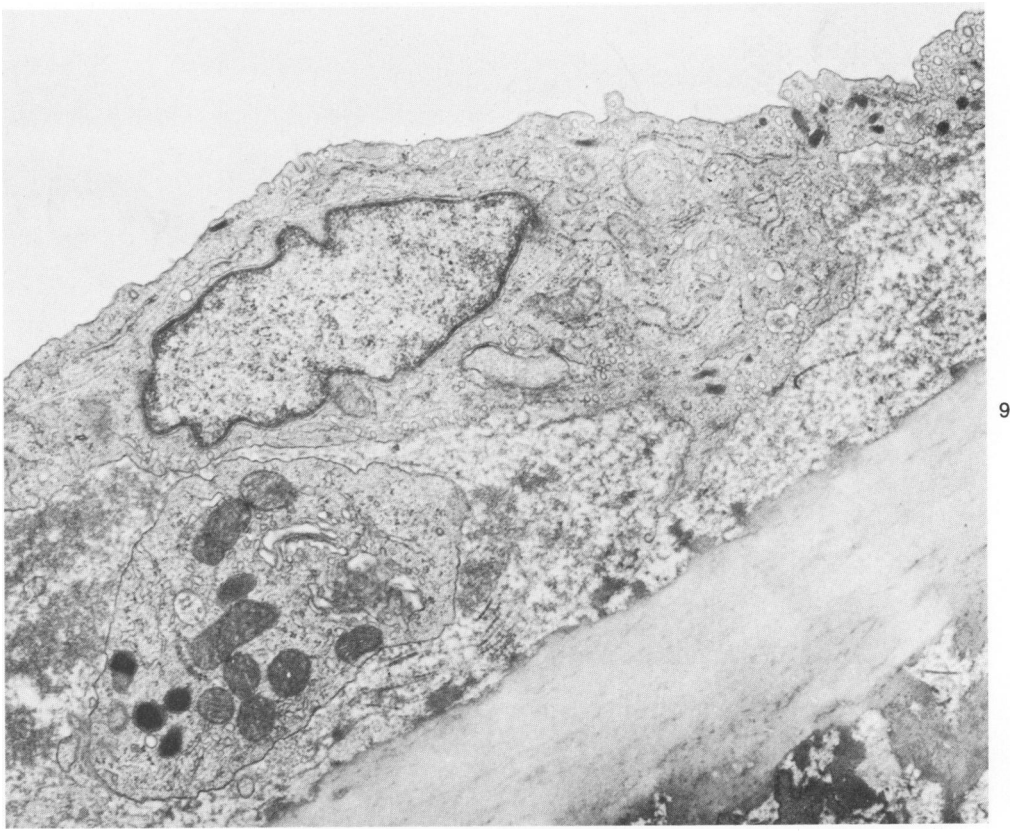

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Figure 8—*En face* preparations of aortic endothelial cells incubated with (**A**) normal human serum on a preparation from a control rat; (**B, C,** and **D**) AAA serum on a preparation from a control rat (**B**), from a hypertensive rat at 7 days (**C**), and from a hypertensive rat at 40 days after aortic ligature (**D**). The first incubations were followed by fluorescein-conjugated antihuman IgG. No fluorescence is present after incubation with normal human serum (**A**); after incubation with AAA, a barely visible fluorescence is noted at the periphery of the cells in control animals (**B**), while bright fluorescence is present in most of the endothelial cells of hypertensive animals at 6 days (**C**); the fluorescence is weaker again at 40 days (**D**). (×400) **Figure 9**—Intima of the thoracic aorta in a uninephrectomized rat receving the Na-rich diet for 40 days. The cytoplasm contains the usual organelles, but it does not contain microfilament bundles. The subendothelium is thickened and contains basement-lamina-like material. (×13,000)

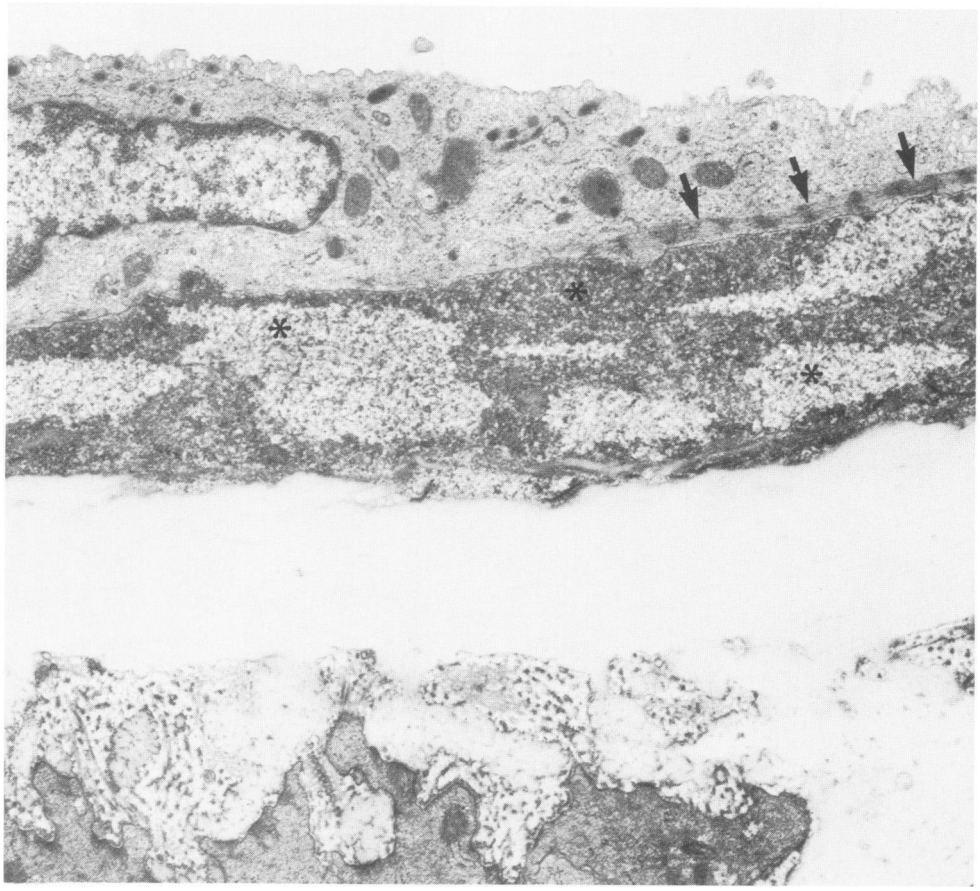

Figure 10—Intima of the thoracic aorta in a uninephrectomized rat drinking 0.9% NaCl and treated with DOCA for 10 days. The cell is hypertrophic. The cytoplasm is well preserved; in addition to the usual organelles, it contains a bundle of microfilaments (*arrows*). The subendothelium (*) is thick and contains HRP reaction product. (×13,000)

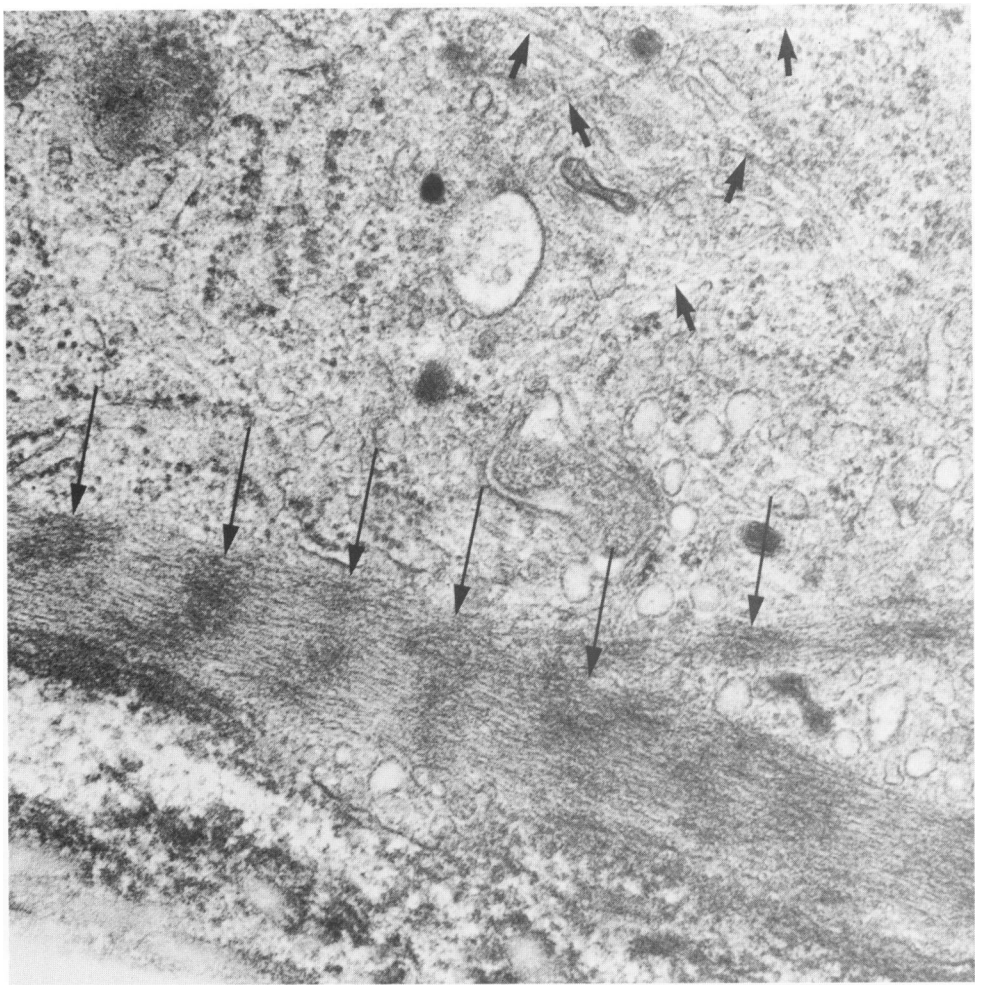

Figure 11—Detail of the cytoplasm of an aortic endothelial cell in a uninephrectomized rat treated with DOCA for 10 days. At the cell periphery, a bundle of microfilaments with electron-dense bodies scattered in between (*long arrows*) is visible. Note the cytoplasmic microtubules (*small arrows*). (×49,200)

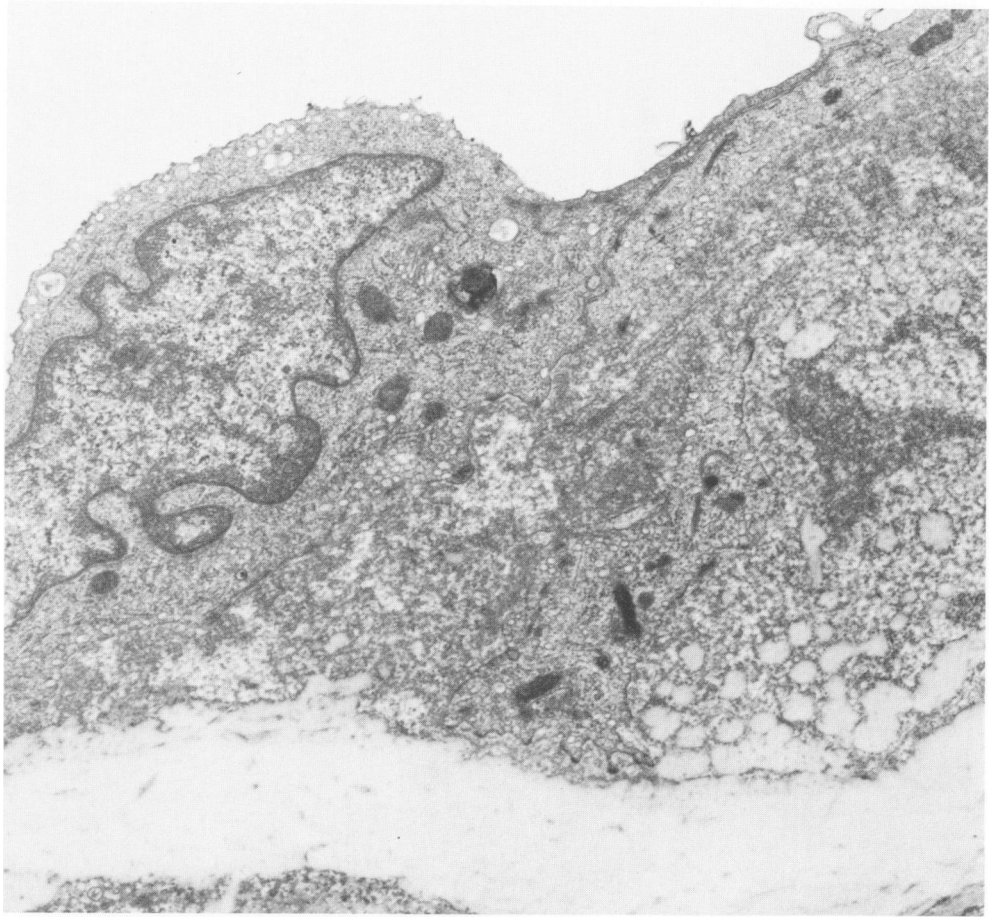

Figure 12—Intima of the thoracic aorta of a rat 40 days after unilateral nephrectomy, 0.9% NaCl in drinking fluid, and DOCA. The endothelial cell is hypertrophic; the subendothelial layer is thickened and contains basement-lamina-like material, collagen, and elastic fibers. (×13,000)