Medial Aortic Lesions in Rats with Metacorticoid Hypertension

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IT IS ACCEPTED today that the pathologic consequences of human and experimental hypertension are primarily found in the arterioles. Reports of aortic lesions associated with hypertension [1-8] have not found their way into accepted medical thinking. Textbooks of pathology, or even a recent and most comprehensive monograph on hypertension,[9] do not mention the reports cited. Yet the subject is obviously important since, as Freis [10] has pointed out, hypertension appears to be the single most important known factor to be influential in the course and even the genesis of atherosclerosis.

The present experiment was designed to study the ultrastructure of the aorta in rats with metacorticoid hypertension.[11] Under the conditions of this experiment, medial as well as intimal lesions were produced. This report concerns itself with an ultrastructural study of the medial lesions.

Materials and Methods

Seventy male Sprague-Dawley rats, 30 days old, were divided in 2 groups. Group 1, composed of 20 rats, remained as an untreated control. Fifty rats, Group 2, were given a subcutaneous implant of a 25 mg pellet of pure desoxycorticosterone acetate (DOCA). Both groups were offered, starting on Day 0, ad libitum, 0.86% Na Cl as drinking fluid. On Day 90, when all the animals in Group 1 and 28 animals in Group 2 were alive, tap water replaced saline as the drinking fluid. Blood pressures were taken at irregular intervals (every 2–3 weeks) using a photoelectric tensometer (Metro Industries, Long Island City, New York) and no anesthesia. Control and treated animals were sacrificed at irregular intervals from Day 100 up to Day 300 when the experiment was terminated.

At autopsy, samples of heart, kidneys, mesentery, and diaphragm were taken and processed by conventional histological techniques. Samples of ascending and descending aorta were fixed in s-collidine buffered osmium and flat embedded in Epon. Thick (1–2 μ) sections were cut with glass knives and stained with toluidine blue. Thin sections were cut with diamond knives, stained with uranyl acetate and lead citrate,[12] mounted on formvar coated copper grids and examined on an RCA Electron Microscope.

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Supported in part by Grant HE-10471 from the National Heart Institute, US Public Health Service, and by a grant from the New Jersey Heart Association.

Accepted for publication Nov 1, 1969.

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Results

Blood Pressure

The animals in Group 2 were hypertensive by the fifth week, their blood pressure averaged 172 mm Hg. At the time when 0.86% NaCl was substituted for tap water, their blood pressure averaged 175 mm Hg. The animals in Group 1 had averages lower than 150 mm Hg. These results were anticipated since the course of metacorticoid hypertension is predictable.[11,14]

Gross Observations and Light Microscopy

In confirmation of a previous report [1] the animals in Group 2 had lesions in the muscular arteries in the heart, kidney, and mesentery. These lesions have not been properly described and no attempt to do so was made in the present study. The aortae in the animals of Group 2 appeared fairly normal, perhaps firmer and slightly dilated compared to those of the controls. Thick sections stained with toluidine blue were of much more value than conventional paraffin sections. It was found very useful to take color photographs of thick sections which could be projected repeatedly. Comparison between animals could be easily made after a study of the photographs, and areas with lesions could easily be identified for sectioning. In most of the animals, areas of metachromasia were scattered throughout the media (Fig 1 and 2). These areas were confined to the space between elastic lamellae. Inside the area, no cells, 1–3 fragments of cells, or small cells with rounded nuclei were found. The media appeared otherwise normal; no adventitial thickening or cellular infiltration was seen.

Electron Microscopy

Medial changes in the aortae of the rats in Group 2 were striking. As suspected from observations made with the light microscope, the changes were focal but widespread since most of the blocks showed them. All the components of the media, namely smooth muscle, collagen, ground substance, elastin, and elastic lamellae were affected, but not to the same extent. These changes are illustrated in Fig 3–12.

Alterations in smooth muscle cells varied from changes in shape and in cytoplasmic organelles to cellular death. Basic changes in the configuration of cells, easily identifiable as smooth muscle cells, can be seen in Fig 5 and 7, and particularly in Fig 6 which illustrates cells with striking cellular processes reminiscent of pseudopodia. Cells were observed with a cytoplasm filled up with an imposing array of organelles (Fig 8).

These cells were identified as smooth muscle cells by two criteria:

First, their location between elastic lamellae away from both intima and adventitia, and second, the presence of intracytoplasmic filaments. In some instances, cells (Fig 11 and 12) were identified by using the first criterion only. Cellular death is documented (Fig 9–12) by two observations: The absence of cells between elastic lamellae, and the presence of cellular debris. Necrosis occurred simultaneously in separate foci of the same block and affected one or several cells, but always within the boundaries of a pair of elastic lamellae. These foci of necrosis never exceeded the space formerly occupied by 10 cells.

As cells departed from normal shape, the amount of collagen and ground substance about them increased. Intracellular vacuoles, probably dilations of the endoplasmic reticulum, contained wisps of material similar to that seen extracellularly (Fig 7). When necrosis occurred, the space formerly occupied by cells became loosely filled with collagen and ground substance (Fig 8–10, 12). Conversely, elastin appeared to be present in inverse relationship to the number of cells (Fig 10–12) and to the number of cellular organelles (Fig 8). The surface of elastic lamellae facing areas of necrosis assumed a flat, smooth contour as compared with the surface facing normal cells. A few negative observations are noteworthy. No evidence of calcification was seen; no proliferation of the adventitia or invasion of the media by fibroblasts or inflammatory cells was found; vasa vasorum were not identified; there was no proliferation of cells in the adventitia.

Discussion

This investigation demonstrates the presence of lesions in the aortic media of rats with metacorticoid hypertension.

Corticoid hypertension of the malignant type was first induced by Selye, Hall, and Rowley with a combination of daily injections of DOCA, 1% NaCl (as drinking fluid), and unilateral nephrectomy.[13] Salgado and Mulroy[11] reported several modifications to the original technique, namely: implantation of a single pellet of DOCA instead of daily injection, lowering of the concentration of NaCl in the drinking fluid from 1% to 0.86% and omission of the nephrectomy. With these modifications, strikingly long survival in a substantial number of animals displaying sustained hypertension was achieved, particularly if 0.86% NaCl was offered to the animals for only a few months. It appears unlikely to this writer that animals with such long survival are in chronic renal failure, but, nevertheless, in future experiments, this point will be explored. Others[15] have shown that rats with metacorticoid hypertension have normal blood cholesterol levels.

Studies of aortic intimal lesions in hypertension are numerous and Still [7,8] has used electron microscopy in an elegant demonstration of the focal intimal thickenings of large arteries. Medial aortic lesions have been reported in hypertension produced in rats with severe obstructive nephropathy,[2] chronic renal failure,[5] and antikidney serum nephritis.[6] Basically, the lesions are similar in the three situations and consist of extensive cellular necrosis and calcification with destruction of elastic lamellae. Although these lesions appear very different from the ones described here, it would be of great interest to study the early phase of this type of lesion to see if the differences persisted.

Ashworth and Haynes[1] reported degeneration of cells and collapse of elastic lamellae in the aortic media of hypertensive patients in addition to a lymphocytic infiltration and changes in the vasa vasorum. They suggested that the lesions were due to constriction of vasa vasorum. This interesting suggestion has received recent support in the finding of Wilens, Malcolm and Vazquez [16] of aortic medial necrosis in the dog after interfering with vasa vasorum by ligation of intercostal arteries. Since, in confirmation of a previous report,[17] no vasa vasorum were found in the aorta of the rat in this study, it would appear unlikely that such a mechanism, essentially ischemia, would be responsible for the lesions.

There is evidence that blood cells, macrophages, cell fragments, and fibrin accumulate in the intima of the renal artery and aorta in hypertension.[7,8,18] Esterly and Glagov [18] have suggested that if the vessel is permeable to formed elements, it might be permeable to other blood constituents. If this is the case, and it is reasonable to suppose that it is, then it could be assumed that as a result of increased permeability in hypertension, smooth muscle cells are surrounded by blood constituents in excess of normal under conditions of normotension. The cells then react to the changed milieu by changing from a contractile cell to a secretory cell, hence the accumulation of ground substance and collagen. Necrosis could represent exhaustion of overstimulated cells. This explanation does not take into consideration other possibilities such as a direct effect of DOCA or of some abnormal constituent of the blood present in hypertensive animals or smooth muscle cells.

Whatever the explanation may be, the smooth muscle cells and their reactions play a central role in the pathogenesis of the lesions described here as they do in atherosclerosis,[19,20] aortic coarctation,[21] and lathyrogenic injury.[22]

It is striking to notice the similarities of the lesions reported here with the ones found with the light microscope by Manley and Burns [23]

in the iliac arteries in 20 cases of dissecting aneurysm. The significance of this similarity is difficult to evaluate at the present time since dissecting aneurysms have not been reported in DOCA hypertension and none was found in the present study. An ultrastructural study of the aorta in human cases of dissecting aneurysm is warranted by these results.

Summary

The fine structure of the aortic media of rats with chronic metacorticoid hypertension was studied. Under the conditions of the experiment, focal but widely distributed changes were found affecting smooth muscle cells, elastic lamellae, elastin, and ground substance. Smooth muscle cells showed changes in shape and increased numbers of intracytoplasmic organelles. These changes were accompanied by an increase in the amount of ground substance and collagen around the smooth muscle cells. Foci of necrosis of smooth muscle cells, involving 1–10 cells, were also detected. Elastin was diminished or absent in these areas of necrosis, but there was no calcification. It is suggested that normal structure and metabolism of smooth muscle cells plays a decisive role in maintaining the structural integrity of elastin.

References

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The excellent technical assistance of Miss Diana Costello and Mr. Cesar Fernandez and the secretarial assistance of Mrs. Elfi Kopczik is gratefully acknowledged.

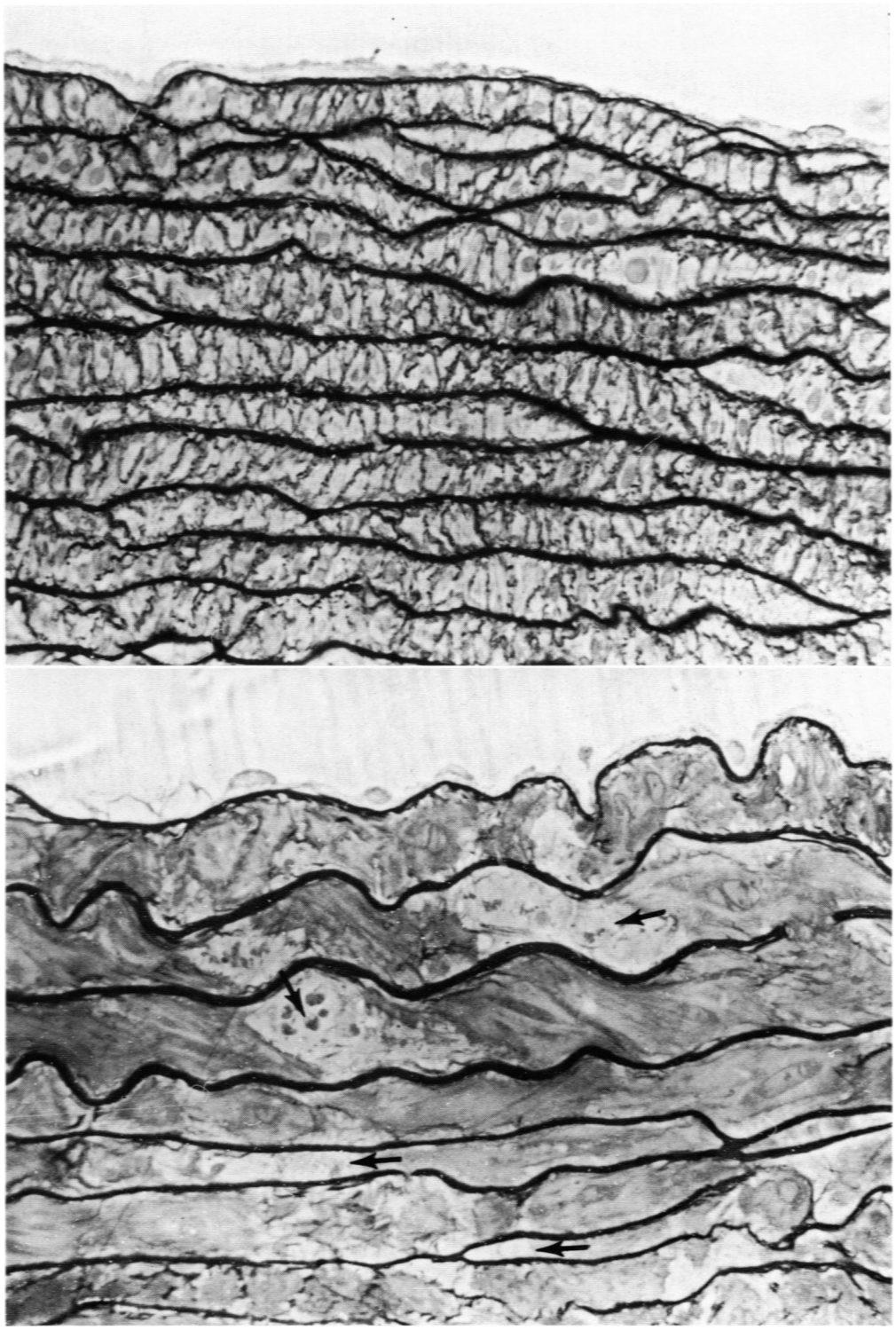
[Illustrations follow]

Legends for Figures

All micrographs were made from sections cut from aorta fixed in s-collidine buffered osmium tetroxide and embedded in Epon. Thick sections for light micrography were stained with toluidine blue. Thin sections for electron micrography were stained with uranyl acetate and lead citrate.

Fig 1. *(upper)* **Aorta of control rat (224 days) showing orderly arrangement of cells, nuclei, elastin, and collagen between elastic lamellae. Phase contrast and a dark blue filter were used to bring up desired detail. × 750.**

Fig 2. *(lower)* **Aorta of hypertensive rat (224 days). Nuclei and cells are arranged disorderly. Areas of metachromasia *(arrows)* in which smooth muscle cell(s) are absent. Phase contrast and a light blue filter were used to bring up desired detail. × 1250.**



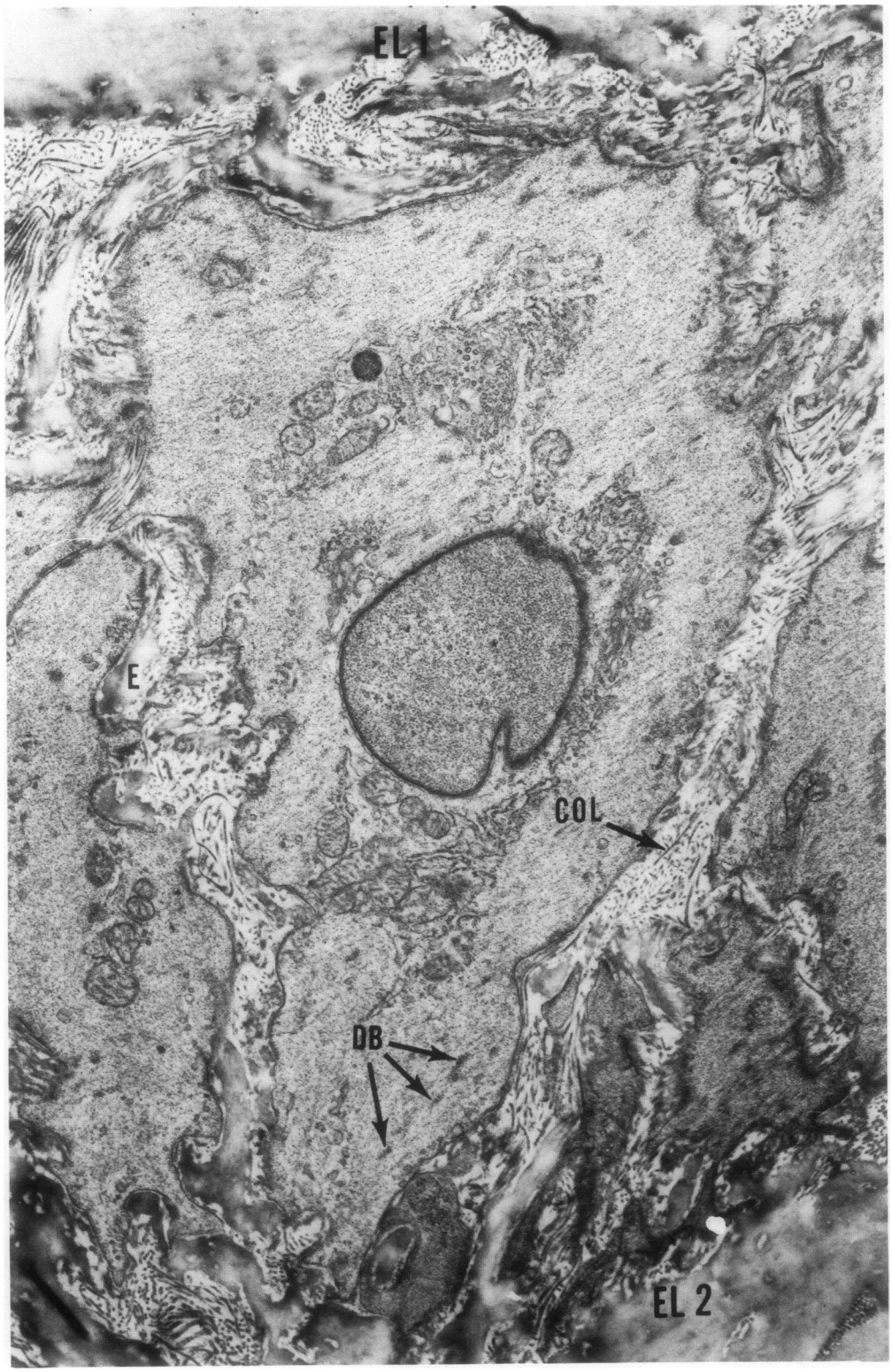


Fig 3. Portion of aortic media of control rat (224 days) bounded by two elastic lamellae *(EL1, EL2)* showing appearance of smooth muscle cells in control rats. *COL* indicates collagen; *E*, elastin; and *DB*, cytoplasmic dark bodies. × 9000.

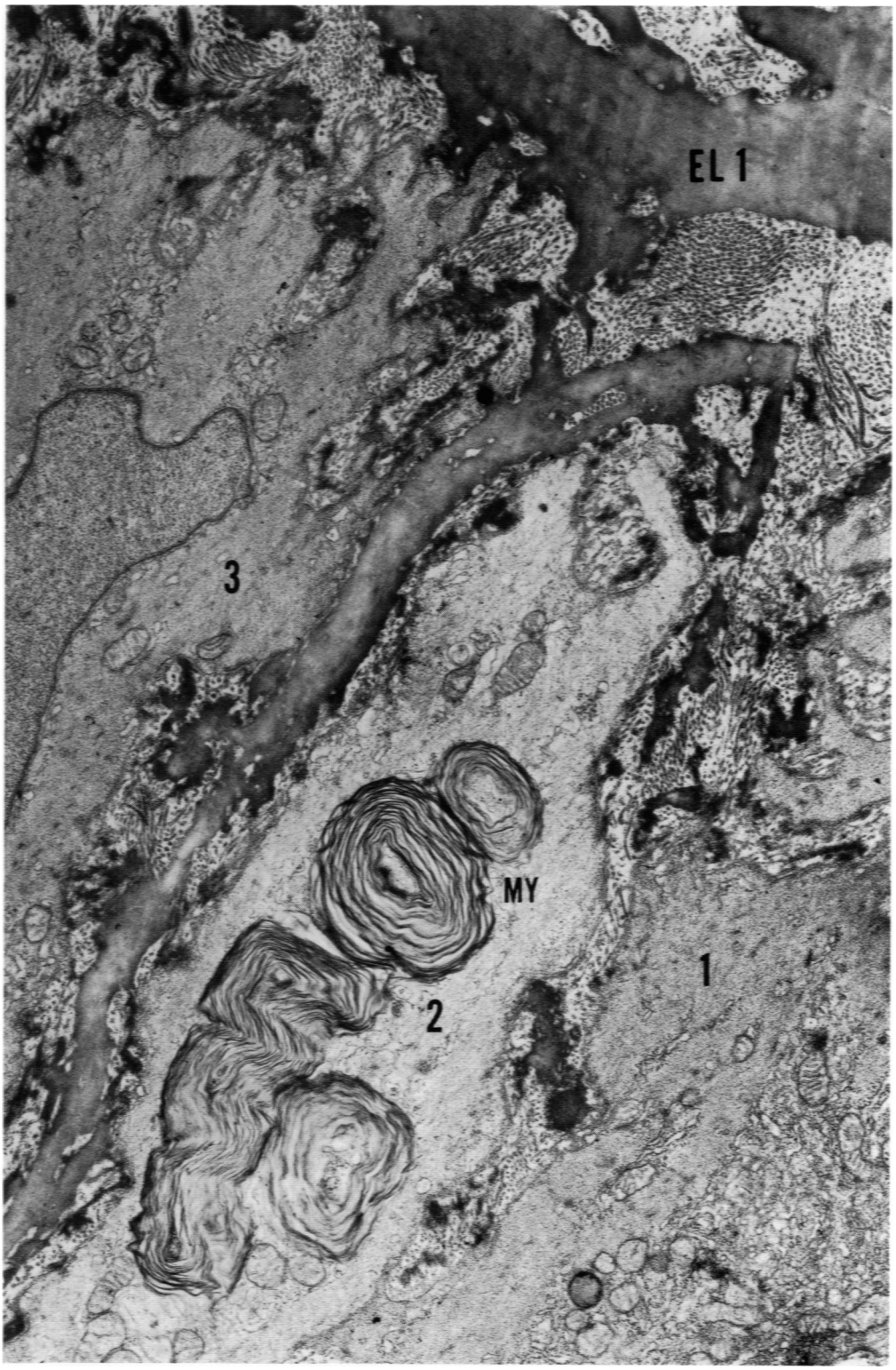


Fig 4. Portion of aortic media of hypertensive rat (169 days) showing increased numbers of organelles in one smooth muscle cell *(1)*, myelin figures *(MY)* in another *(2)*, and fairly normal appearance of another *(3)*. *EL1* indicates elastic lamella. × 9000.

Fig 5. *(upper)* Portion of aortic media of hypertensive rat (169 days) bounded by two elastic lamellae *(EL1, EL2)* showing three smooth muscle cells *(1, 2, 3)* of irregular contour and with many thin cell processes *(arrows). COL* indicates collagen; *E*, elastin. × 8700.

Fig 6. *(lower)* Portion of aortic media of hypertensive rat (169 days) bounded by two elastic lamellae *(EL1, EL2)* showing smooth muscle cells *(1, 2)* of bizarre shape and with numerous thin processes *(arrows).* Note elastin *(E)* fragments, very few collagen bundles *(COL)* and very abundant flocculent material, interpreted as ground substance *(GS).* × 7500.

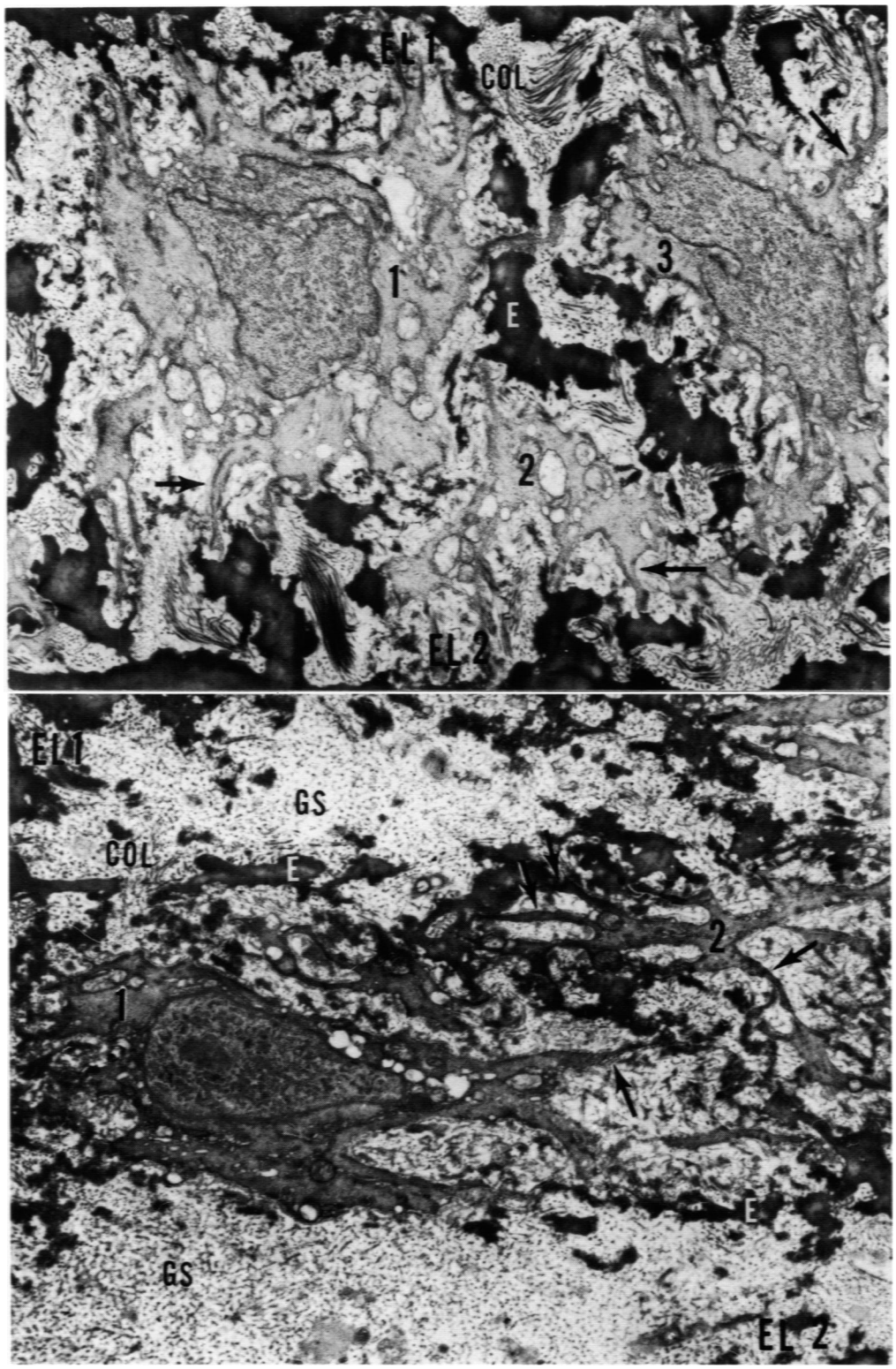

EL 1
COL
1
E
3
2
EL 2
EL 1
GS
COL
E
2
1
E
GS
EL 2

Fig 7. Portion of aortic media of hypertensive rat (169 days) bounded by two elastic lamellae *(EL1, EL2)*. Smooth muscle cell shows vacuoles *(arrows)* filled with flocculent material. Note serrated appearance of cell borders. *COL* indicates collagen; *DB*, cytoplasmic dark bodies. × 6000.

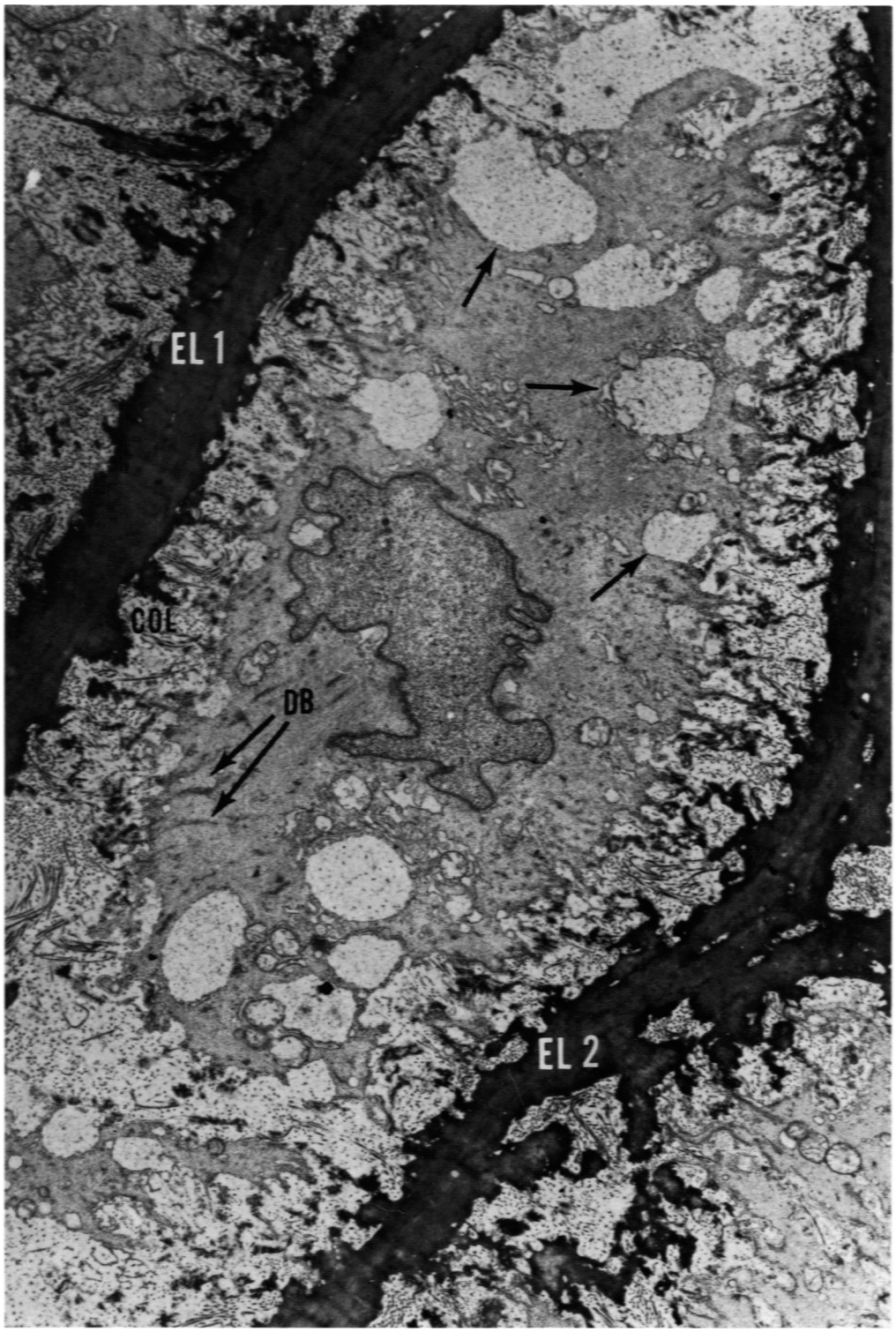
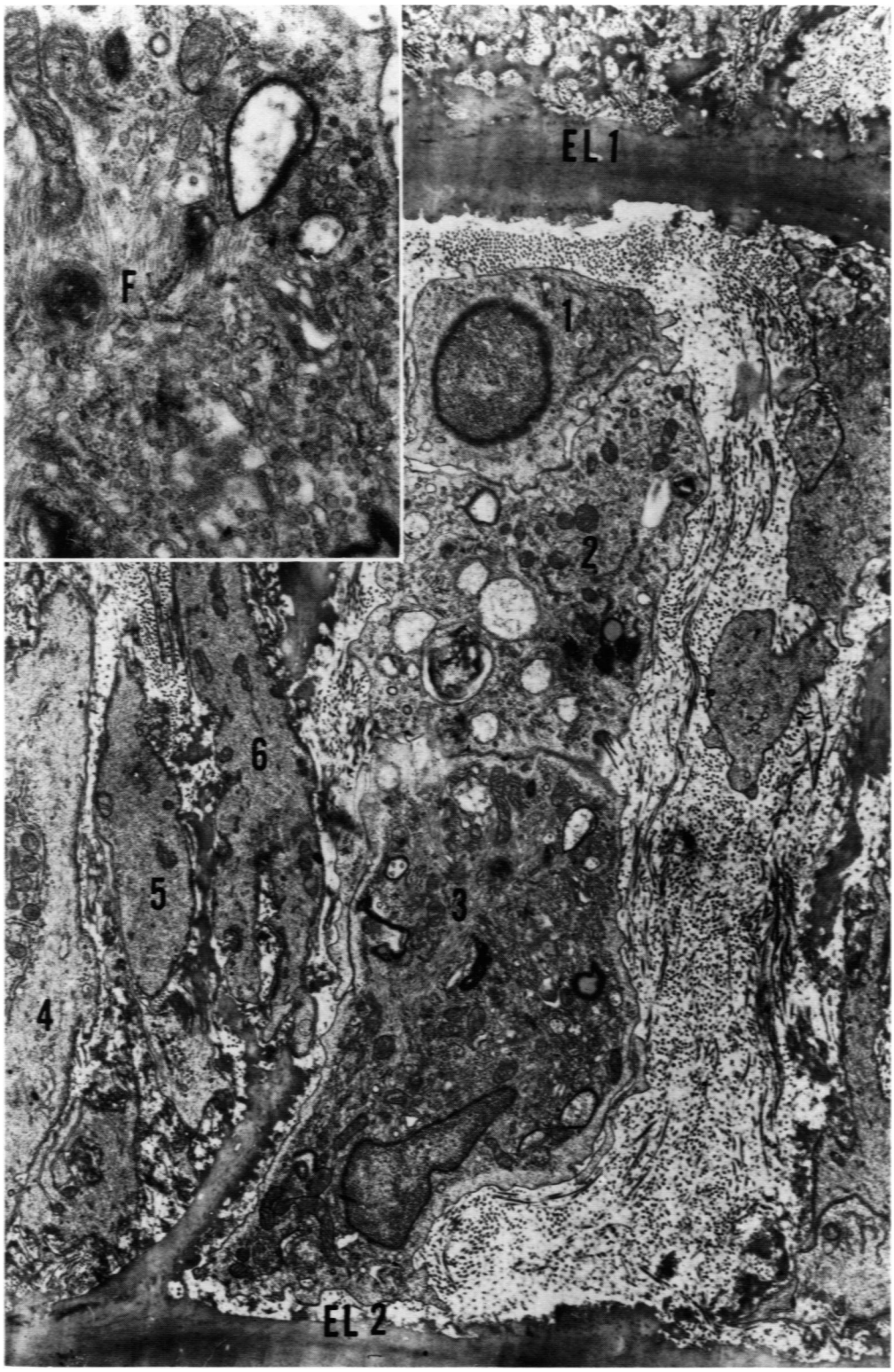

EL 1
COL
DB
EL 2

Fig 8. Portion of aortic media of hypertensive rat (169 days) bounded by two elastic lamellae *(EL1, EL2)* showing smooth muscle cells *(1–6)*. Cells labelled *1, 2, 3* show excessive number of organelles suggesting participation in formation of ground substance and collagen seen about them. Elastin is absent around cells *1–3* and present around cells *4–6*. Note also flat appearance of border of elastic lamella facing cells *1–3*. × 6000. **Inset.** High power of cell labelled *3* showing parallel arrays of filaments *(F)*. × 24,000.


F
EL 1
1
2
6
5
3
4
EL 2

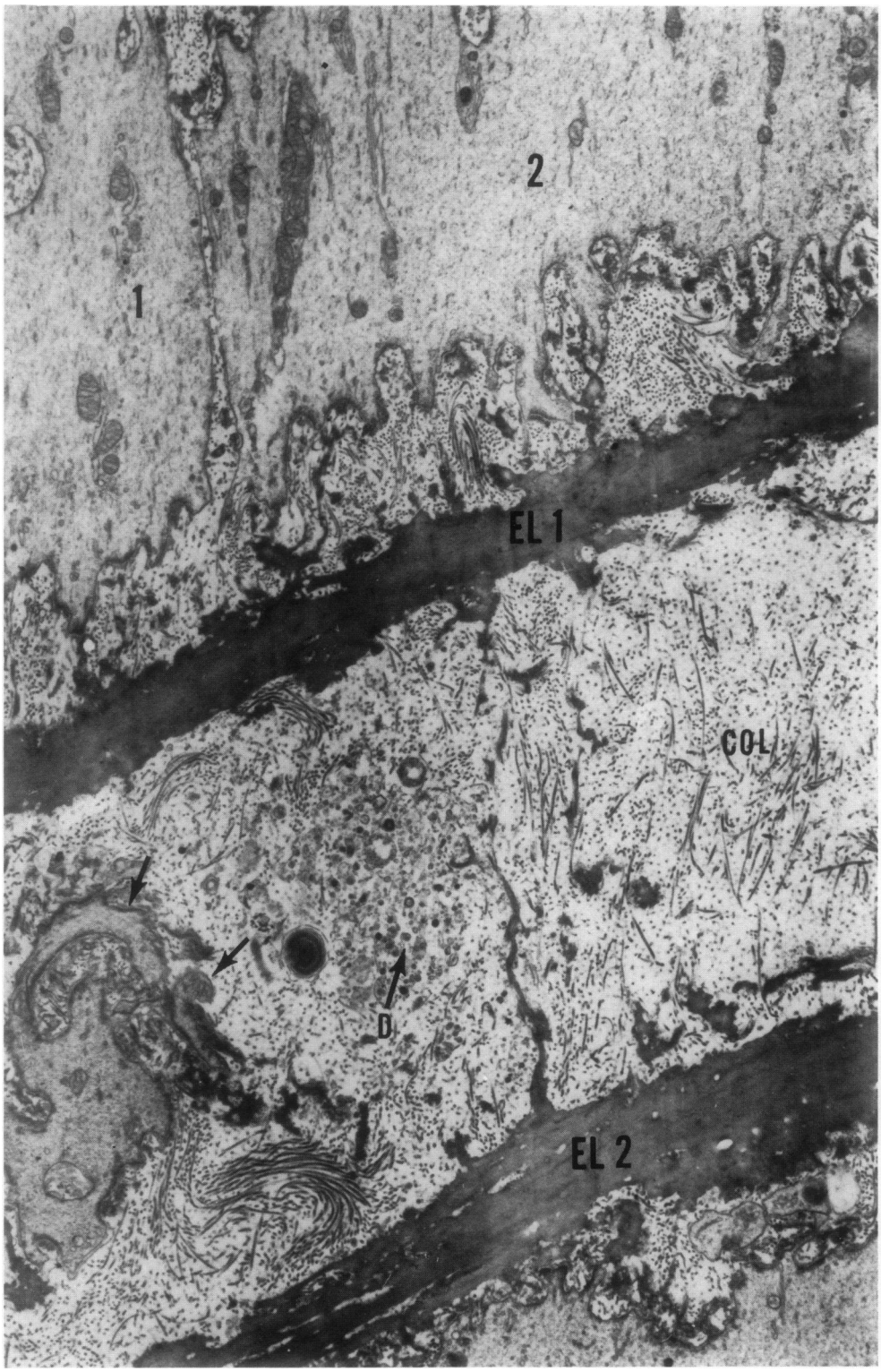


Fig 9. Portion of aortic media of hypertensive rat (224 days) showing two elastic lamellae *(EL1, EL2)*. Note two fairly normal smooth muscle cells *(1, 2)* and in lower part of micrograph cell, processes *(arrows)* of one or more cells, cellular debris *(D)*, and collagen *(COL)*.

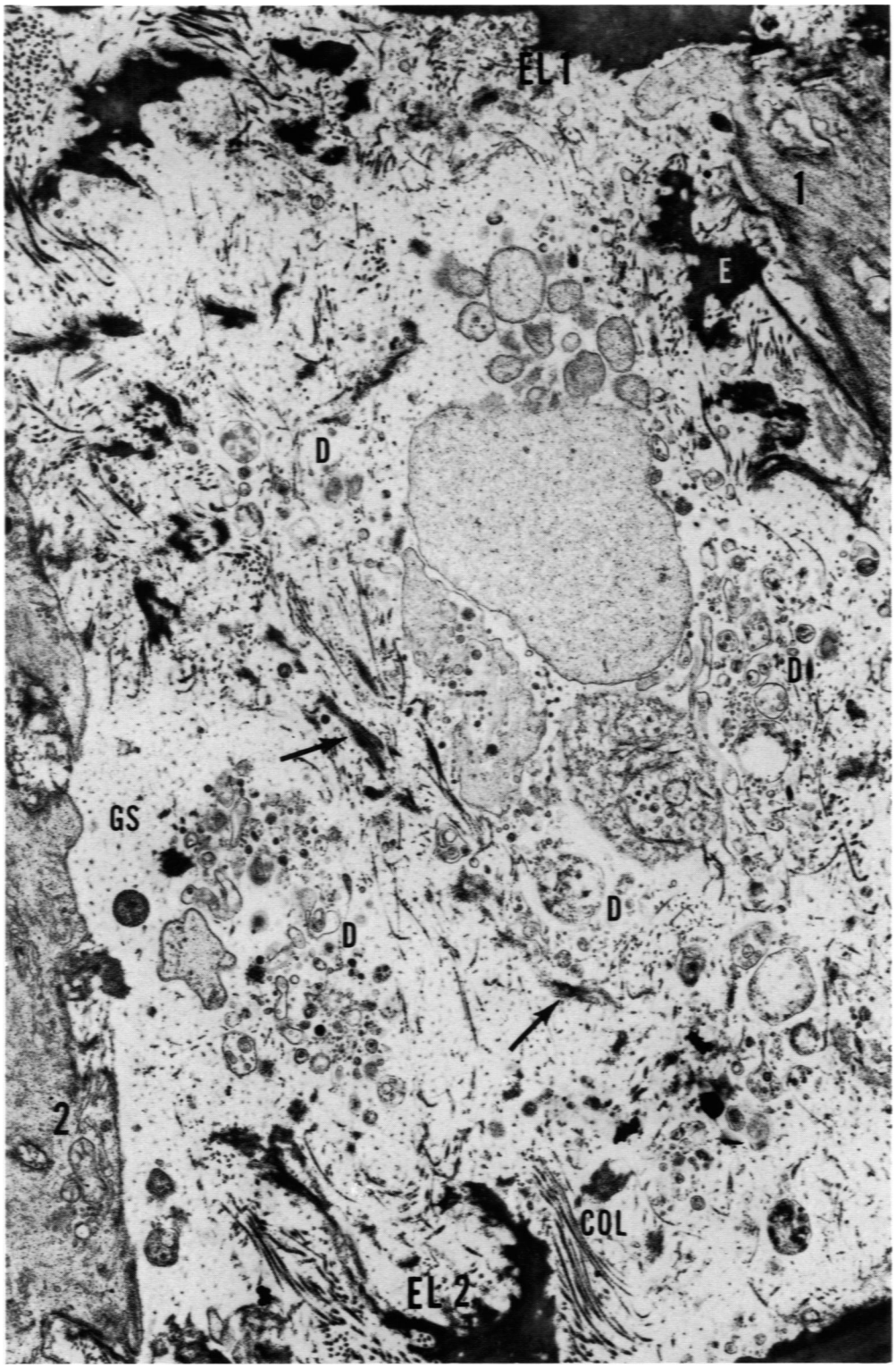


Fig 10. High power of area similar to center of Fig 9 showing elastic lamellae *(EL1, EL2)*, smooth muscle cells *(1, 2)*, cellular debris *(D)*, elastin *(E)*, collagen *(COL)*, and ground substance *(GS)*. Elastin *(arrows)* in center is sparse and relatively light. × 13,500.

Fig 11. Portion of aortic media of hypertensive rat (224 days) showing many smooth muscle cells *(1–7)* of fairly normal appearance. Cells 8 and 9 are modified smooth muscle cells showing atypical cytoplasm. Cellular debris *(D)*, thick bundles of collagen *(COL)*, and elastin *(E)* are present. Side of elastic lamellae facing acellular portions of media is regular in contour. × 5800.

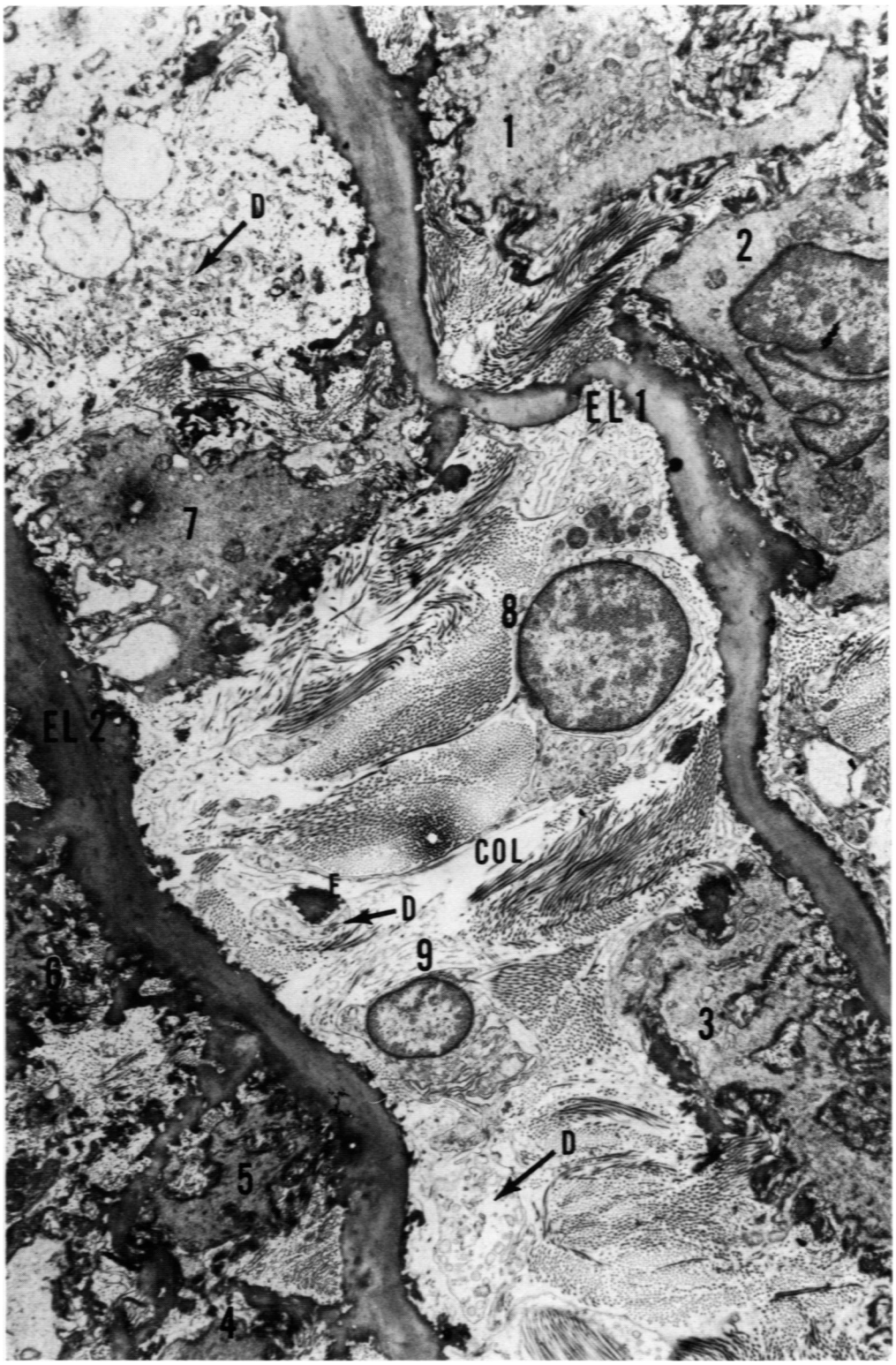
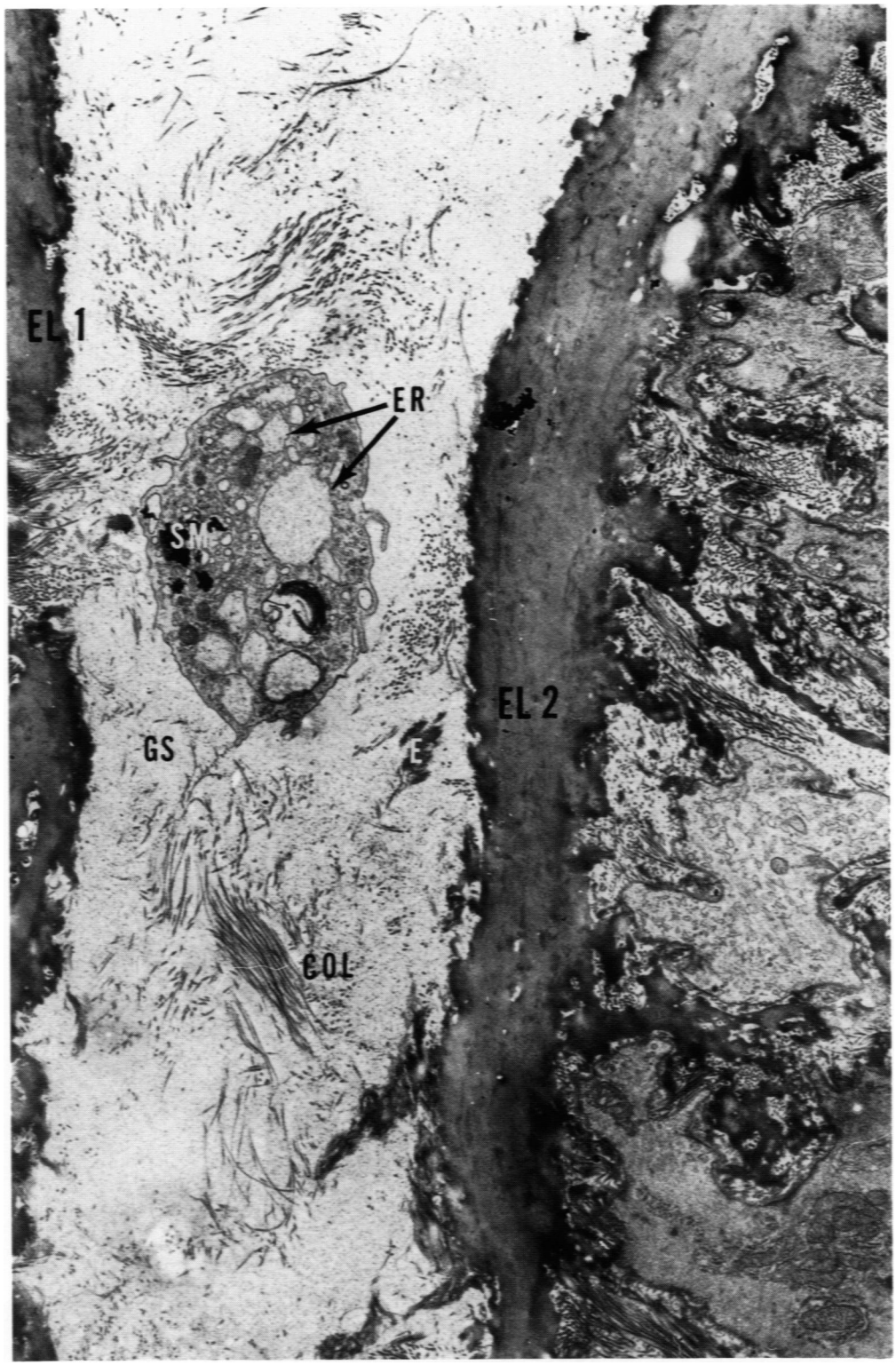

1
2
D
EL 1
7
8
EL 2
COL
F
D
9
6
3
D
5
4

Fig 12. Portion of aortic media of hypertensive rat (224 days). Single cell *(SM)* between elastic lamellae *(EL1, EL2)* is interpreted as modified smooth muscle cell and shows prominent dilated endoplasmic reticulum *(ER)* filled with flocculent material similar to ground substance seen extracellularly *(GS)*. Note uniformity of elastic lamellae border facing acellular areas. Note also how scanty is elastin *(E)* in same areas. In a low power micrograph of the field depicted above, it was calculated that 8 cells have disappeared from central area. × 7600.


EL 1
ER
SM
EL 2
GS
E
COL