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Fibrinoid Degeneration and Increased Vascular Permeability Induced by Renal Lysosomal Contents

An Electron Microscopic Study on Coronary and Cerebral Arteries of Rats

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Summary. The effect of the lysosomal contents of hog kidney cortex, especially of the fraction not bound by concanavalin A (Fraction A) on the permeability of the coronary and cerebral arteries of rats was studied ultrastructurally using H.R. peroxidase. This fraction was devoid of renin activity by bioassay.

The coronary arteries of the experimental rats displayed fibrinoid degeneration: e.g., degeneration and disappearance of medial smooth muscle cells and deposition of electron dense materials containing fibrin. A large amount of reaction product of peroxidase was present in the subendothelial space and media where fibrinoid degeneration was evident. Transendothelial passage of the marker occurred by both junctional and vesicular transport. There was no evidence of separation or discontinuity of the endothelial cells. Occasionally, increased permeability of the intima was noted in the coronary arteries without medial damage. By contrast, neither fibrinoid degeneration nor increased permeability was noted in the cerebral arteries. The difference in the response of the two arteries seems attributable to the barrier effect of cerebral arterial intima.

Key words: Fibrinoid degeneration – Vascular permeability – Renal lysosomal contents – Coronary artery – Cerebral artery.

Introduction

In previous papers (Nakamura et al., 1975, 1978), we reported that the lysosomal contents (Ly-C) of hog kidney cortex induced fibrinoid degeneration of the small arteries and arterioles of the pancreas, mesentery and heart in bilaterally nephrectomized rats and increased vascular permeability of rabbit skin. Both

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fractions separated by applying Ly-C to a concanavalin A affinity column; an unbound fraction (Fraction A) devoid of renin and a renin rich bound fraction (Fraction B) induced fibrinoid degeneration of the arteries and increased vascular permeability of rabbit skin (Nakamura et al., 1978).

Although it seemed evident that renin was not required to induce fibrinoid degeneration, changes of permeability and the development of fibrinoid degeneration had been evaluated in different animal models, the former in rabbit skin and the latter in small arteries of the rat. To correct this shortcoming, the effect of Ly-C, especially of fraction A having no renin activity, on coronary and intracranial cerebral arteries of the rat was studied ultrastructurally using H.R. peroxidase to evaluate changes in permeability.

Materials and Methods

The procedure for obtaining renal lysosomal contents has been reported elsewhere (Nakamura et al., 1975) but is outlined briefly herein.

I. Preparation of Subcellular Fractions

a) Fractionation by Differential Centrifugation. Fresh hog kidneys were obtained from a slaughter house and refrigerated. Dissected cortical tissues were minced, homogenized in cold 0.45 M sucrose-0.68 mM EDTA solution, and centrifuged for 5 min at 1,000 g. The supernatant was centrifuged for 5 min at 7,000 g yielding a precipitate containing lysosomal and mitochondrial fractions. To disrupt lysosomal membranes, this was suspended in 0.05 M sucrose-0.076 mM EDTA (pH 7.0) for 2 h and centrifuged for 30 min at 105,000 g. The supernatant contained lysosomal contents (Ly-C).

b) Purification of Vasoactive Substances from Ly-C by Concanavalin A Affinity Chromatography. When Ly-C is applied to concanavalin A affinity column, renin is absorbed completely. An unbound fraction (A) is obtained by eluting with 0.02 M phosphate buffer-1 M NaCl (pH 7.0). The bound fraction (B) was separated from the column with methyl-α-D-glucoside. Renin activity of Fraction A and B was measured by the method by Conradi et al. (1969), yielding the results previously reported (Nakamura et al., 1978).

II. Observations of Vascular Lesions and Vascular Permeability

A total of 23 male Wistar rats weighing 170–200 g were bilaterally nephrectomized. Six hours later, Fraction A or B was injected intraperitoneally. Fourteen rats were injected with Fraction A (in terms of protein, 5 mg was administered to 10 rats and 10 mg to 4 rats, dissolved in 3 ml of saline). Four rats were given 5 mg of Fraction B, and for controls, 5 rats were injected with 0.25 M sucrose.

Blood pressure was measured just before sacrifice using a tail-pulse pick up method. Furthermore, mean carotid arterial blood pressure of 2 rats administered fraction A or B (2 rats each) in the conscious unrestrained state was recorded continuously for 18 h using a polygraphic recorder with a pressure transducer (Kai et al., in press).

Fifteen minutes before sacrifice 24 h after nephrectomy H.R. peroxidase (10 mg/100 g body weight, dissolved in a total of 1 ml of isotonic saline) was injected into the tail vein of 4 rats administered Fraction A, 2 rats administered Fraction B and 2 rats administered sucrose.

For electron microscopic study, tissue blocks were removed from the right and left intramuscular coronary arteries, and basilar arteries and intracerebral small arteries. They were fixed in 3% glutaraldehyde buffered with 0.1 M cacodylate for 5–6 h at room temperature and then washed overnight at 4° C in 0.1 M cacodylate buffer. After postfixation with 1% OsO_4 buffered with

0.1 M cacodylate for 1.5 h, they were dehydrated in graded ethanol, treated with propylene oxide, and embedded in EPON 812.

Prior to osmification, however, glutaraldehyde fixed tissue blocks from rats injected with H.R. peroxidase were sliced by the vibratome (Oxford) at a thickness of 30 μ, and incubated for 15 min at room temperature in 10 ml of 0.05 M Tris-HCl buffer (pH 7.6), containing 0.01% H_2O_2 and 5.0 mg of 3-3′ diaminobenzidine tetrahydrochloride.

Thin sections were cut on a LKB ultrotome, stained either with uranyl acetate and lead citrate or lead citrate only and examined with a JEM-100 C electron microscope.

For light microscopic study, the brain, heart, pancreas and mesenterium were removed and fixed in 10% formalin and stained with H & E and PAS.

Results

I. Blood Pressure

Systolic blood pressure of the rats administered sucrose or Fraction A just before sacrifice ranged from 100 to 120 mm Hg, but that of the rats administered

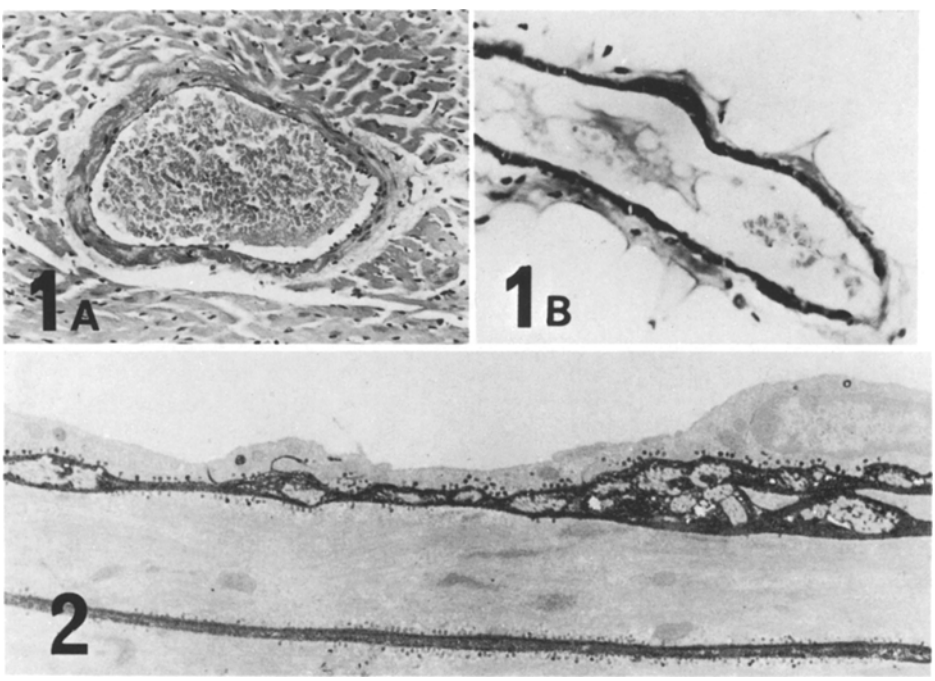

Fig. 1. A Right coronary artery of the rat 18 h after administration of Fraction A. PAS staining. ×112, PAS positive materials are deposited in the media in granular or nodular fashion. **B** Mesenteric artery of the rat 18 h after administeration of Fraction A. PAS staining. ×310. PAS positive materials are deposited in almost the entire circle of the vessel

Fig. 2. Coronary artery of the rat 18 h after administration of sucrose. ×4,000. The internal elastic lamina is frequently disrupted. The reaction product of peroxidase is present in the caveolae and vesicles of the endothelial cells, interendothelial and subendothelial spaces. In the media, the reaction product is present in the caveolae of the smooth muscle cells and intercellular space

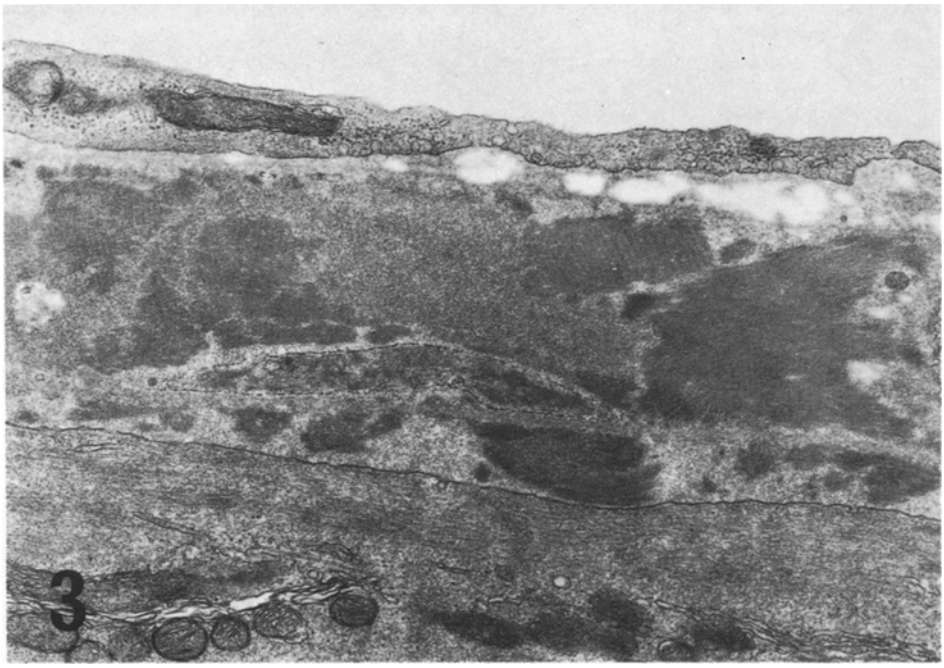

Fig. 3. Coronary artery of the rat 18 h after administration of Fraction A. ×10,000. Electron dense materials deposited in the inner layer of the media show transverse striation of 380–390 Å. The plasma membrane of a smooth muscle cell presents multiple dotted line-like interruptions; electron dense materials are present in the cytoplasm

Fraction B ranged from 150 to 160 mm Hg. When Fraction A was injected into 2 conscious bilaterally nephrectomized rats, mean blood pressure increased gradually from 100 to 120, and from 100 to 130 mm Hg during the first hour respectively, and then gradually decreased to the control level within 18 h.

The injection of Fraction B (2 rats) induced a prompt rise of the mean blood pressure from 80 to 160 and from 75 to 165 mm Hg, this remained elevated for 1 to 2 h and persisted in excess of 150 mm Hg for 18 h.

II. Light Microscopic Findings

a) Coronary, Pancreatic and Mesenteric Arteries. In these arteries of the rats administered Fraction A, fibrinoid degeneration of small arteries and arterioles was observed. Affected areas were focal, ranging from segmental to circumferential involvement and were characterized by eosinophilic and strongly PAS positive materials deposited in granular or nodular pattern (Fig. 1 A). In severe cases, PAS positive deposits involved the entire wall of the vessels homogeneously (Fig. 1 B). Most endothelial cells seemed to be intact; there was slight perivascular oedema. Lesions were somewhat more frequent and severe in the right than in the left coronary artery.

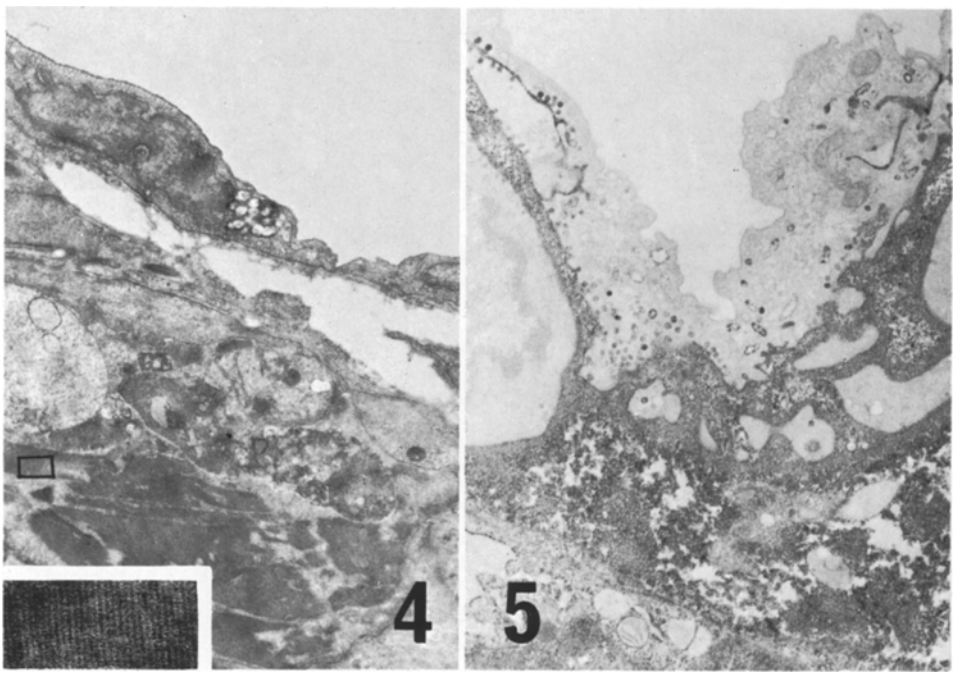

Fig. 4. Coronary artery of the rat 18 h after administration of Fraction A. ×3,500 Electron dense filamentous materials deposited in the intercellular space of the media where vacuoles and membraous materials are also present. A smooth muscle cell shows increased density of cytoplasm and small membranous materials. The endothelial cells show increased numbers of caveolae and vesicles. There is no separation of the intercellular junctions nor discontinuity of the endothelial cell lining. Inset: Higher magnification of the inset shows transverse periodicity of 230 Å. ×48,000

Fig. 5. Coronary artery of the rat 18 h after administration of Fraction A. ×4,200. A large amount of the reaction product of peroxidase is present in the media. It is also found in the caveolae and vesicles of the endothelial cells and smooth muscle cells, and in the interendothelial spaces

Fraction B induced more frequent and severe fibrinoid degeneration in the examined arteries than Fraction A. The arteries of the control rats administered sucrose were histologically intact.

b) Cerebral Arteries. Cerebral arteries of all experimental rats were entirely normal.

III. Electron Microscopic Findings

1. Fine Structure and Permeability Pattern of the Coronary Artery

a) Control Rats Administered Sucrose. Endothelial cells contained a moderate number of caveolae and vesicles. There were microfibrils and dense granular materials in the subendothelial space. The internal elastic lamina was frequently

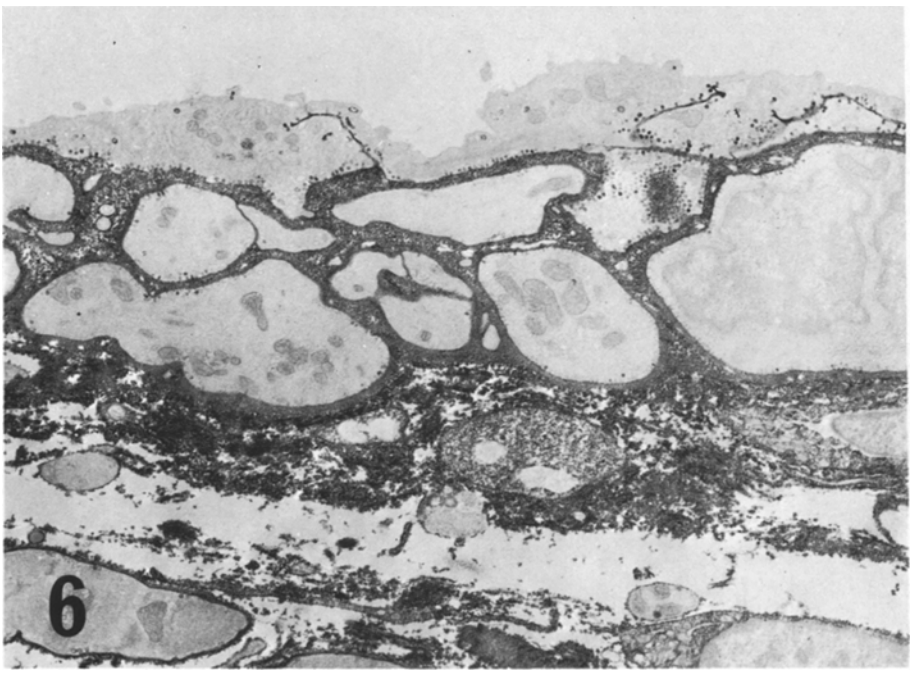

Fig. 6. Coronary artery of the rat 18 h after administration of Fraction A. ×3,200. A large amount of reaction product of peroxidase is present in the subendothelial space and intercellular space of the media where necrosis and degeneration of smooth cells are noted. The reaction product is present in the interendothelial spaces, and caveolae and vesicles of the endothelial cells and smooth muscle cells

disrupted, disappearing gradually with decrease of luminal size. Medial smooth muscle cells were well formed and intact; collagen fibers and elastic fibers were observed in the intercellular space. In the intima, reaction product of peroxidase was present in the caveolae and vesicles of the endothelial cells and interendothelial spaces (Fig. 2). A small amount of the reaction product was identified in the subendothelial space and intercellular space of the inner layer of the media.

b) Experimental Rats Administered Fraction A. Areas with fibrinoid degeneration by light microscopy showed varied medial alterations by electron microscopy. Electron dense materials were observed in the intercellular spaces and in the cytoplasm of medial smooth muscle cells (Figs. 3, 4). Deposits varied in shape and size and were homogeneous, granular or filamentous in character. Occasionally a distinct transverse striation of 230–250 Å periodicity, and sometimes longer (380–390 Å) periodicity could be demonstrated (Figs. 3, 4).

The cytoplasmic membrane of the smooth muscle cells was disrupted and indistinct (Figs. 3, 4). The cytoplasm was homogeneous or finely granular con-

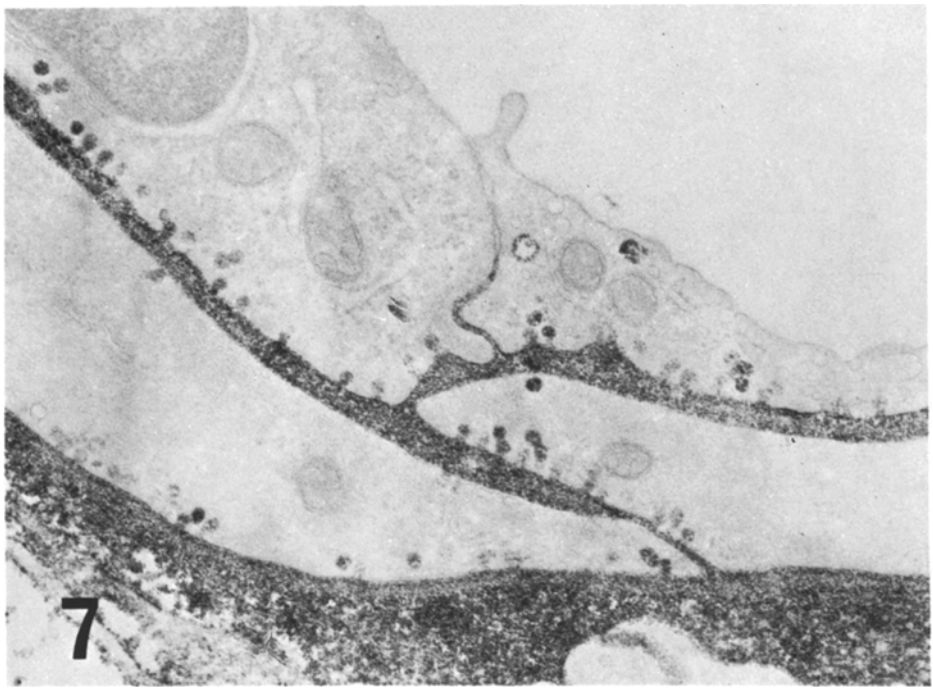

Fig. 7. Coronary artery of the rat 18 h after administration of Fraction A. ×14,500. A large amount of the reaction product is present subendothelially in the intima and media. Cellular degeneration is not evident

taining scanty organells, some of which were extruded into the intercellular space (Fig. 4).

Variously sized vacuoles occupied the cytoplasm. Cellular organelles of severely damaged smooth muscle cells were depleted and were occasionally replaced by electron dense material. The endothelial cell lining was intact although cytoplasmic vesicles and vacuoles were increased in number (Fig. 4). There was no evidence of junctional widening or endothelial cell contraction. No vesicular open channels were observed in the endothelial cell lining (Simionescu et al., 1975).

An abundance of peroxidase had accumulated in necrotic and degenerate medial foci (Figs. 5, 6). In the intimal lining, reaction product was present in endothelial vesicles and intercellular junctional spaces (Figs. 5, 6). Reaction product was demonstrable in caveolae of intact smooth muscle cells (Fig. 5) and occasionally was present well beneath the endothelium unassociated with degeneration of smooth muscle cells (Fig. 7).

c) Experimental Rats Administered Fraction B. The changes in the coronary arteries were indistinguishable from those of the rats which received Fraction A.

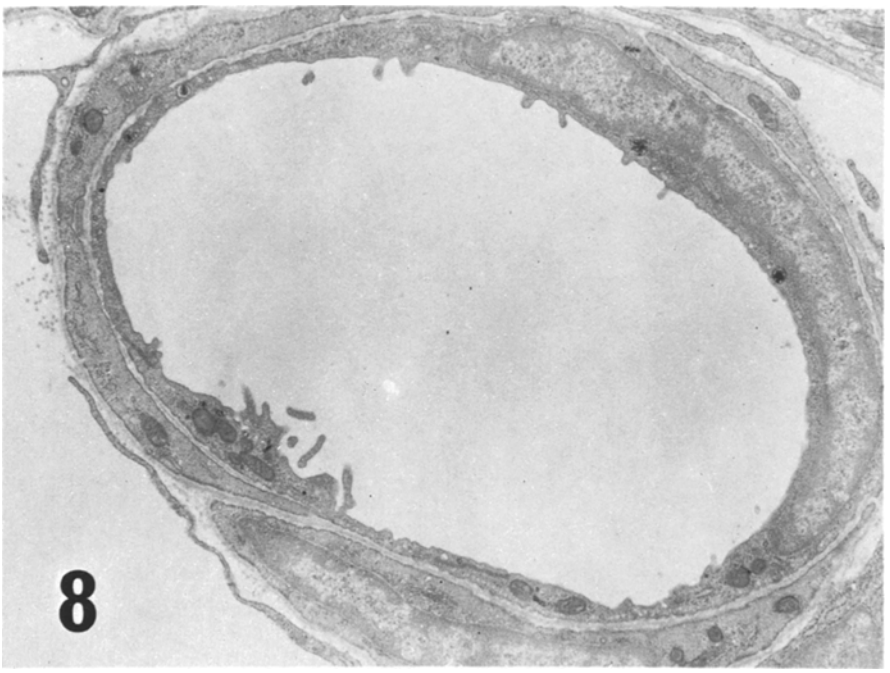

Fig. 8. Intracranial artery of the rat 18 h after administration of Fraction A. × 10,000. The endothelial cells show villous projections of the luminal cytoplasm; the medial smooth muscle cells are unaltered. Reaction product is present only in the sparse caveolae and vesicles of the endothelial cells

2. Fine Structure and Permeability Pattern of the Cerebral Artery

a) Control Rats Administered Sucrose. Cerebral arterial endothelial cells contain many fewer caveolae and vesicles than those lining of the coronary artery. The endothelium is closely applied to the internal elastic lamina except for an indistinct space containing coarse fibrillar basement membrane like material. Subendothelial cells were infrequent and were only observed at branching sites. The medial smooth muscle cells were structurally normal. After exposure to H.R. peroxidase reaction product was identified in a few endothelial caveolae and vesicles. Neither the junctional spaces nor the subendothelial space were stained.

b) Experimental Rats Administered Fraction A. The cerebral arteries in these rats were indistinguishable from those of control rat (Fig. 8).

c) Experimental Rats Administered Fraction B. Again the cerebral arteries were indistinguishable from either those of the controls or those of the Fraction A group.

Discussion

Winternitz (1940) demonstrated that kidney homogenate contained substances which induced fibrinoid degeneration of small renal arteries. Since then, multiple studies have been conducted using renal extracts or subcellular fractions to identify the factors involved (Asscher and Anson, 1963; Giese, 1963; Cuthbert et al., 1966; Cuthbert and Peart, 1970; Shimomura, 1971). There is still uncertainty as to the exact intracellular origin of these substances in the kidney, e.g., lysosomal or microsomal, as well as to their relation to renin or other vasopressor (Cuthbert et al., 1966; Shimomura, 1971; Nakao et al., 1966; Kira et al., 1968; Onoyama et al., 1971). In previous studies (Nakamura et al., 1975, 1978), we found that the lysosomal contents (Ly-C) of rat and hog kidney cortex induced fibrinoid degeneration of the mesenteric, pancreatic and coronary arteries of rat and increased vascular permeability of rabbit skin. Two fractions separated by applying Ly-C to a concanavalin A affinity column also induced fibrinoid degeneration of coronary, pancreatic and mesenteric arteries of rats and increased vascular permeability of the rabbit skin, although one fraction (A) was devoid of renin activity and did not have strong pressor activity (Fraction B).

Further studies seemed appropriate to ascertain whether the vascular lesions induced by extracts of the kidney were related to permeability changes in the same arteries. Few studies have been concerned with this critical point (Shimomura, 1971). The present investigation of the influence of the two Ly-C fractions on the development of vascular lesions clearly demonstrates that increased permeability was the primary event. This was observed regularly with fibrinoid degeneration but occasionally was noted in its absence justifying the conclusion that increased permeability preceded the development of fibrinoid degeneration of the media. Presumably this resulted from the precipitation of fibrin from the plasma insudate by thromboplastic constituents of damaged smooth muscle cells.

With experimental hypertension, increased permeability of the renal, pancreatic and mesenteric arteries of rats has been attributed to endothelial defects that ranged from widening of endothelial cell junctions to loss of one or more endothelial cells (Wiener et al., 1969; Giacomelli et al., 1970). Recently, transendothelial channels in the capillary endothelial cells were proposed by Simionescu et al. (1975). However, such findings were absent from the coronary artery in the present study and it is presumed that plasma constituents enter the intima and media in an increased amount through the physiological pathways-intercellular junctions and vesicular transport (Hüttner et al., 1970). There was no way of determining which of these routes predominated.

As to the contribution of bilateral nephrectomy to the development of vascular lesions, Churg and Paterson (1963) reported that angionecrosis occurred in the rats surviving 3 days or longer after bilateral nephrectomy but never in those dying in the first 48 h. At the ultrastructural level, Goldby and Beilin (1972) reported no vascular lesions in the intestine 60 min after bilateral nephrectomy. Recently, using electron microscopy, Eto et al. (1970) observed fibrinoid degeneration of arterioles of the intestinal submucosa of rats 20–24 h after

bilateral nephrectomy. However, this study showed neither fibrinoid degeneration nor an increased vascular permeability in the coronary and cerebral arteries of bilaterally nephrectomized rats administered sucrose.

Hypertension must be considered a factor in the development of fibrinoid degeneration following the administration of renin rich Fraction B. Fraction A produced a slow, mild and transient pressor response, distinctly different from Fraction B in intensity and duration (Kai et al., in press). Nakao et al. (1966) demonstrated non-pressor substances capable of producing vascular injury independent of renin in the ischaemic kidney cortex. The present study similarly showed that fibrinoid degeneration and increased vascular permeability were produced by Fraction A, which is devoid of renin activity by bioassay. Shimomura (1971) also demonstrated fibrinoid necrosis produced by a renal fraction with very weak pressor activity.

The cerebral arteries, contrasting with the other vascular areas, showed neither increased permeability nor fibrinoid degeneration with either Fraction A or B. Previously, we found that permeability of rabbit cerebral artery differs from that of the coronary arteries and aorta. Morphologically cerebral arterial endothelium is characterized by paucity of caveolae and vesicles and by the presence of tight intercellular junctions (Kurozumi, 1975). These are entirely analogous to observations made on capillaries of the brain which account for the blood-brain barrier.

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