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Periarteritis Nodosa in Rats Treated with Chronic Excess Sodium Chloride (NaCl) after X-Irradiation (42548)

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Abstract. Five-week-old male Crj:CD (SD) rats were treated with excess sodium chloride after abdominal X-irradiation. The gastric regions of the rats were irradiated with a total dose of 20 Gy given in two equal fractions separated by 3 days. After X-irradiation, animals were fed a diet containing 10% sodium chloride. Red blood cell anemia appeared 22 weeks after the last irradiation. By gross observation, the mesenteric arteries became reddish in color, and bead- or lead pipe-like nodular thickenings were present. Microscopically, these nodularly thickened mesenteric arteries showed fibrinoid necrosis with massive inflammatory infiltration including eosinophils and neutrophils. In more advanced lesions, elastica interna and externa and medial smooth muscle cells disappeared completely and were replaced by granulation tissue. In old lesions, arterial walls were markedly thickened with fibrous or fibromuscular tissue. These findings were quite similar to those of the human periarteritis nodosa. These arterial lesions could not be found in the rats with X-irradiation only, sodium chloride only, or in nontreated animals. This study demonstrates X-ray-induced, NaCl-promoted periarteritis nodosa-like lesions in rats.

Radiation-induced vascular damage can be a serious problem in clinical radiotherapy (1–8). As has been reported by Dollinger *et al.* (2) subintimal fibrosis of coronary arteries with a narrowing of vascular lumina frequently developed after therapeutic mediastinal irradiation and became a cause of cardiac infarction. In experimental animals, similar findings have been reported by many investigators (9–11). Yang *et al.* (12) investigated the late ultrastructural changes of coronary arteries after fission neutron or ^{60}Co irradiation. They observed that medial smooth muscle cells were first damaged 3 months after irradiation and showed intracellular accumulation of numerous dense bodies and myelin figures. These damaged smooth muscle cells were gradually destroyed and replaced by fibrous connective tissue.

Radiation-induced vascular changes are markedly modified by hypertension (13, 14). Asscher and Anson (13) induced fibrinoid necrosis of spinal arteries in hypertensive rats after irradiation to the spinal cord, while normotensive rats did not show such vascular changes. On the other hand, it is now well known that excess sodium chloride ingestion induces systemic hypertension (15–17). Moreover, Rojo-Ortega *et al.* (18) reported the fact that the incidence of arterial lesions such as fibrinoid necrosis, proliferative endarteritis, and periarteritis nodosa-like lesions was markedly accelerated by the excess sodium chloride ingestion in the rats with experimentally induced malignant hypertension. However, there is little information about the role of sodium chloride in the X-ray-induced vascular lesions.

This study was carried out to investigate the effect of chronic excess sodium chloride ingestion on radiation-induced vascular injury.

Materials and Methods. *A. Animals.* Seventy-seven 5-week-old male Crj:CD (SD) rats, originating from the Charles River SD strain since 1950 (Charles River Japan Inc., Atsugi, Japan), were used in the experiment. They were housed in wire mesh cages, three to five to a cage, and kept in an air-conditioned room.

B. X-irradiation. X-irradiation was according to a method described previously (19–21). Briefly, the rats were anesthetized with nembutal and placed within the X-ray beam. A 0.6-cm-thick lead cover with a hole 1.8 cm in diameter over the gastric region, which was

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settled from the processus xiphoideus, was positioned over the rats. The renal braches were without the field of radiation. They were given a total dose of 20 Gy of X-ray administered in two equal fractions separated by 3 days. Exposure conditions were as follows: 200 kVp; external filter, 0.5 Cu and 1.0 Al; half-value layer, 1.18 mm Cu; and dose rate, 45 R/min, measured with a Radcom 555 dosimeter. A coefficient of 0.95/100 was used in converting roentgen into Grays (=100 rad). The scattered dose to other organs was about 5%.

C. Treatment. After the final irradiation, 19 rats were fed a normal diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) supplemented with 10% NaCl (Wako Pure Chemical Ind., Ltd., Osaka, Japan) throughout the experiment (group 1). The composition of the normal diet is listed in Table I. Eighteen animals were exposed to X-rays only (group 2). Twenty-one animals served as unirradiated controls and were maintained on the NaCl diet (group 3). Nineteen animals were fed a normal diet and not irradiated (group 4). All rats had free access to food and water and were observed for up to 52 weeks after X-ray treatment.

D. Examination of animals. All animals were kept under strict observation. All rats were killed when the experiment was terminated at 1 year after treatment, but some animals were killed before that time, because they were anemic or moribund. Also, those animals that died before they could be sacrificed were autopsied. The major organs such as brain, salivary glands, tongue, lungs, heart, stomach, intestine, pancreas, liver, kidneys, adrenals, prostates, and testicles, and the vascular system including aorta and large and small arteries and veins were examined and prepared for histopathological studies. The tissues were fixed in 10% neutral formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin. As necessary, Azan Mallory, Elastica-Van Giesson, and phosphotungustic acid hematoxylin stains were also utilized (22).

TABLE I. COMPONENTS OF MF DIET PER 100 g

Total protein	24.0 g	Ca	1.10 g		
Total fat	5.1	P	0.83 g	K/Na	2.88
Total ash	6.2	Mg	0.25 g	Ca/P	1.33
Total fiber	3.2	Na	0.26 g	Ca/Mg	4.40
Total calories	359.9	K	0.75 g		
		Fe	0.016 g		
		Al	0.007 g		
		SiO_2	0.24 g		
		Cu	0.74 mg		
		Zn	4.99 mg		
		Co	0.08 mg		
		Mn	4.99 mg		

Results. *A. Macroscopic finding.* Food consumption was the same in rats in any group treated with or without NaCl during the experiment. Water intake and urinary output were higher in NaCl groups than in the other groups. The mean body weight of group 1 at 6 months after the first treatment (418 ± 43 g) was significantly lower than that of groups 2, 3, and 4 (502 ± 45, 517 ± 51, and 536 ± 53, respectively ($P < 0.01$)). The mean durations of study at the time of sacrifice for rats in groups 1 to 4 were 214 ± 46, 328 ± 44, 290 ± 53, and 335 ± 53 days, respectively. There were significant differences in survival between group 1 and groups 2, 3, and 4 ($P < 0.01$). In group 1 red blood cell anemia appeared at 22 weeks after the last irradiation.

The mesenteric arteries were reddish in color and there were bead- or lead pipe-like nodular thickenings on the vessels in group 1 (Fig. 1). A common site of arterial changes was the cranial pancreaticoduodenal artery. The short straight branch of the artery just prior to its penetrance into the duodenal wall was affected. Two animals died by abdominal bleeding because of the rupture of aneurysms in the mesenteric arteries. However, the cranial arteries, celiac arteries, and large arteries near the spine were not affected. Nephrosclerosis was observed in 79% of the animals in group 1 and in 5% of those in group 3; this condition was never found in the animals of groups 2 and 4 (group 1 vs groups 2 to 4: $P < 0.05$). None of these changes were present in groups 2 and 4 (Table II).

B. Microscopic findings. Arterial lesions. Arterial lesions observed in group 1 (X-irradiation and NaCl) were panarteritis resembling periarteritis nodosa. Inflammatory changes of these arterial lesions varied in degree and showed different stages, early to old. The early lesions revealed degeneration of intimal and medial cells with deposition of fibrinoid materials (Fig. 2). Slight inflammatory cell infil-

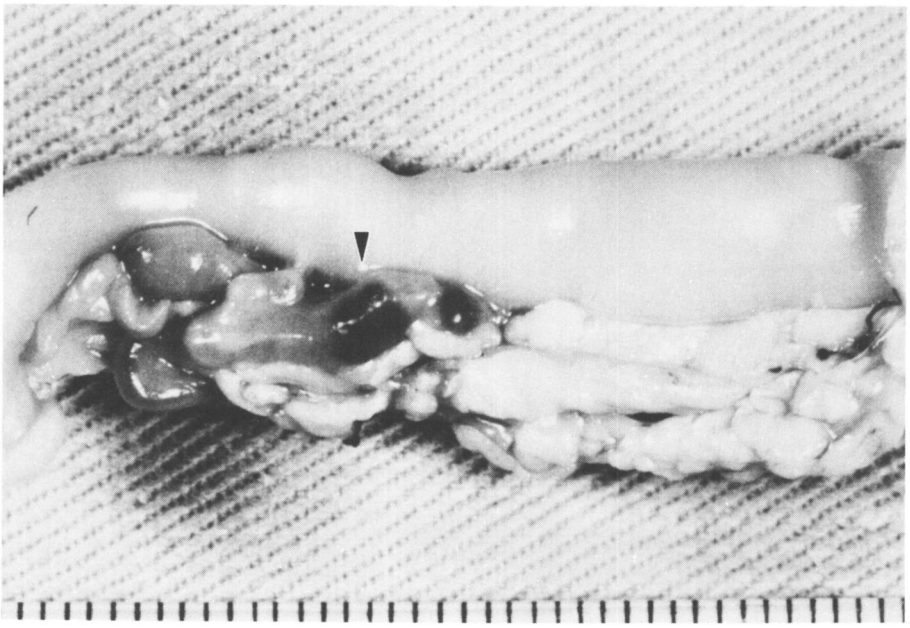

FIG. 1. Lead pipe-like thickening of mesenteric artery (arrow) in the rat treated with chronic sodium chloride after X-irradiation.

tration, including eosinophiles and neutrophiles, was observed in these degenerative areas. Adventitia was thickened with proliferation of mesenchymal cells and inflammatory infiltration. In more advanced lesions, the vascular wall was replaced by granulation tissue with massive inflammatory infiltration (Figs. 3 and 4). The narrowing and obstruction of vascular lumina with thrombosis were frequently observed. In older lesions, the vascular wall was markedly thickened with fibrous or fibromuscular tissue (Fig. 5). Aneurysmal dilatation was frequently observed in the mesenteric arteries showing an old or healed stage. In addition to these periarteritis nodosa-like lesions, subintimal hyaline degeneration of arterioles resembling hypertensive arteriopathy was frequently observed in the heart, lungs, liver, adrenals, kidneys, prostate, and testicles.

The arterial lesions resembling periarteritis nodosa were found in all animals of group 1 (X-irradiation and NaCl) 18 weeks after the last irradiation. However, these lesions could not be observed in the animals of the other three groups (X-irradiation only, NaCl only, and nontreated).

Renal lesions. The kidney which macroscopically showed nephrosclerosis in group 1

TABLE II. NUMBER OF MACROSCOPIC FINDINGS OF ARTERIAL LESIONS AND NEPHROSCLEROSIS INDUCED BY X-IRRADIATION AND/OR NaCl IN SD MALE RATS

Group	Treatment		Effective number	Arterial lesion with nodular thickening of mesenteric artery (%)	Ruptured aneurysms (%)	Nephrosclerosis (%)
	X-ray	NaCl				
1	10 ×2	+	19	16 (84)[a]	2 (11)	15 (79)[a]
2	10 ×2	−	18	0	0	0
3	−	+	21	0	0	1 (5)
4	−	−	19	0	0	0

[a] Was significantly different vs groups 2 to 4 ($P < 0.05$).

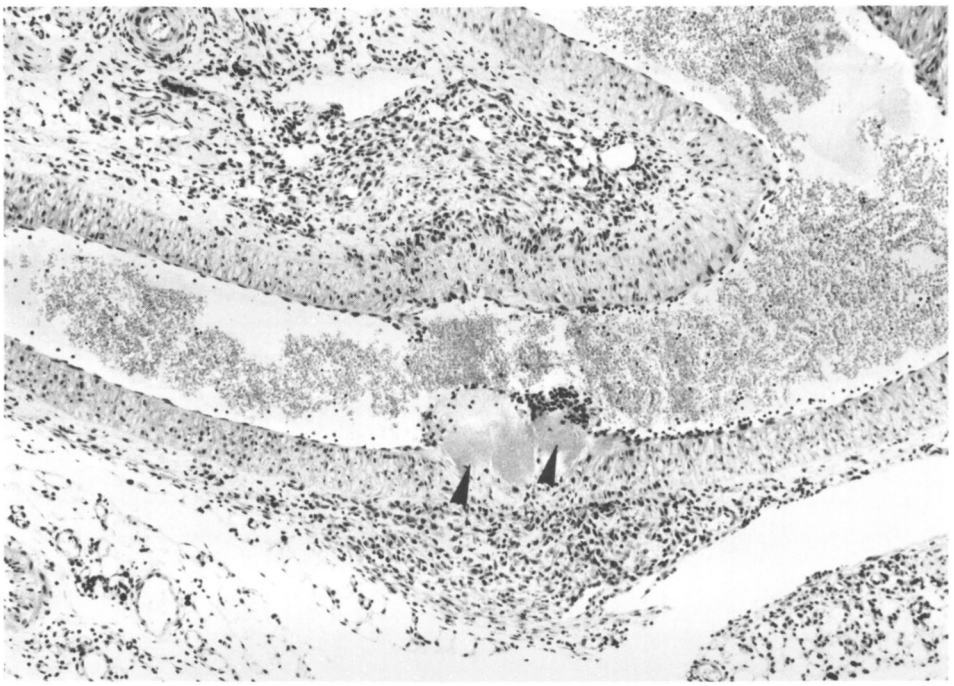

FIG. 2. Early stage of P-N-like lesions in the rat treated with sodium chloride after irradiation. Degenerative change of the intima and media with deposition of fibrionoid materials (arrow) are observed. H-E stain. ×100.

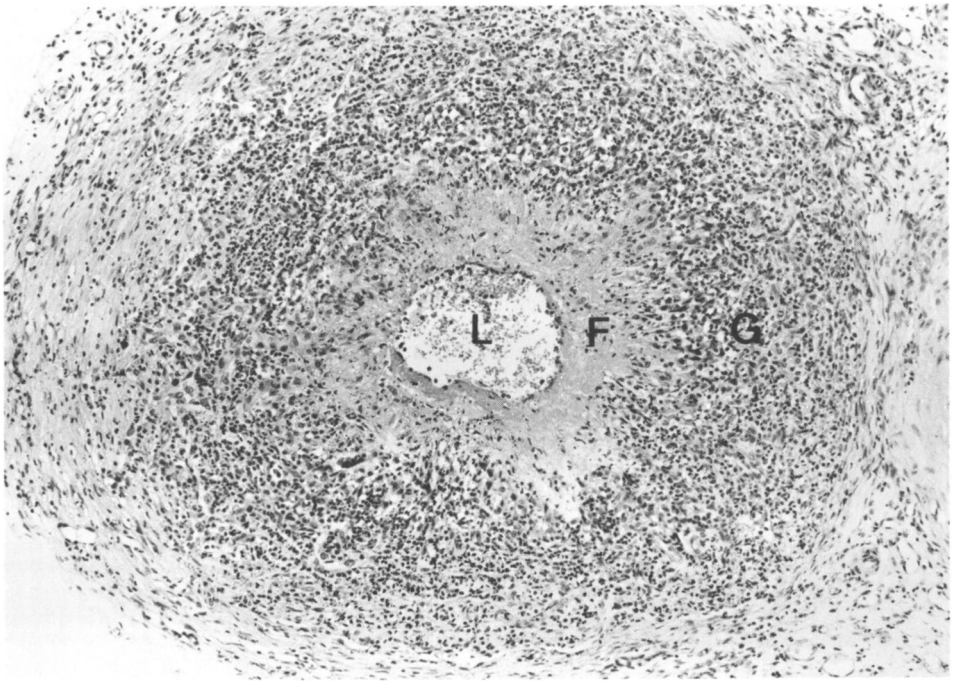

FIG. 3. Advanced stage of P-N-like lesions in the rat treated with sodium chloride after irradiation. Fibrinoid degeneration (F), granulation formation (G), and fibrosis are distinct in the markedly thickened mesenteric artery. L, arterial lumen; H-E stain. ×100.

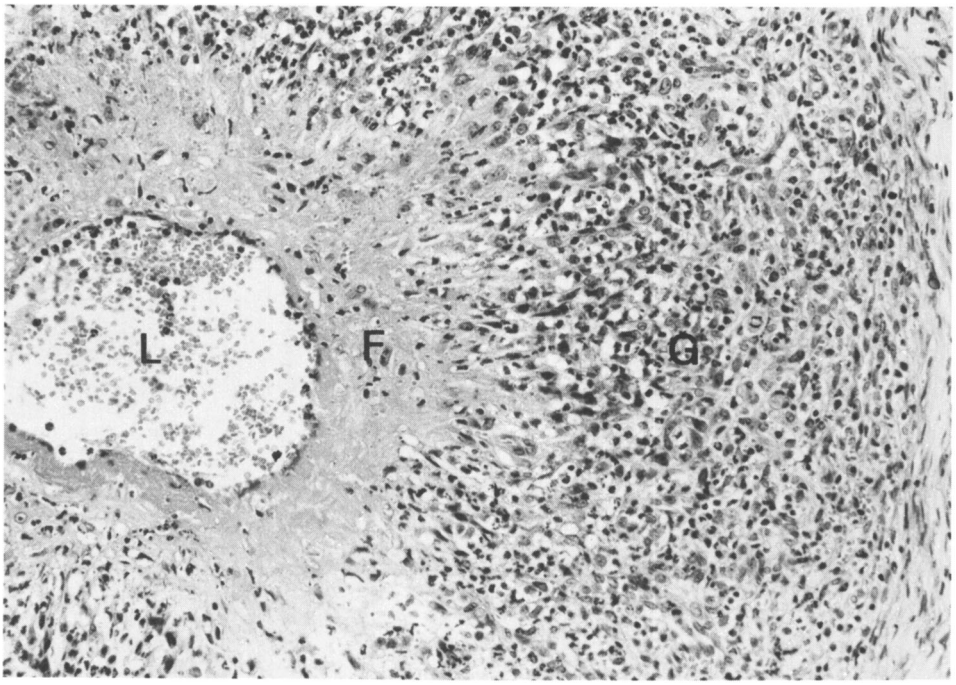

FIG. 4. Large magnification of Fig. 3. Arterial lesions are more distinct. L, arterial lumen; F, fibrinoid degeneration; G, granulation; H-E stain. ×200.

animals revealed intimal thickening of arteries and arterioles with glomerulosclerosis (Fig. 6). Subintimal hyaline degeneration of afferent arterioles of glomeruli was frequently observed. These findings were quite similar to those of hypertensive nephropathy. In the animals of group 3 (NaCl only), on the other hand, renal tubular epithelium was mainly affected, predominantly in the areas of medulla and corticomedullary junction. The affected tubules were grouped and usually composed of 10 to 20 tubules. The tubular epithelial cells of these affected tubules were atrophic and attenuated (Fig. 7). These renal tubular changes were also recognized in the rats of group 1. The renal medulla was more or less atrophic and the renal pelvis was dilated. Renal arteries and glomeruli were well preserved compared to those of group 1 animals. No significant changes could be observed in the kidneys of group 2 (X-irradiation only) and group 4 (nontreated) rats.

Gastrointestinal lesions. Intestinal metaplasia of gastric mucosa appeared in groups 1 and 2. There was no bleeding, erosion, ulceration, or tumors from the gastrointestinal tract.

Discussion. As reviewed by Law (23), radiation-induced vascular injury is divided into three groups, early, intermediate, and late changes. The earliest visible change of blood vessels after irradiation is erythema which appears within a few days. This change is now considered to be a result of the vascular action of histamine-like substances released from dying epithelial cells. Intermediate change is observed at 2 to 6 months after irradiation and characterized by the swelling and sloughing of endothelial cells, thrombus formation, and leakage of plasma proteins into the vascular wall. Late change observed 6 months or later is characterized by a degenerative change in arteries, arterioles, and capillaries. Endothelial proliferation, intimal thickening with a narrowing of the vascular lumens, degeneration and disappearance of medial muscle cells with proliferation of fibrous elements, and damage of the elastic fiber of the vascular wall are characteristic of the late change.

In our present study, arterial lesions resembling periarteritis nodosa were induced in the mesenteric arteries of the rats treated with chronic excess sodium chloride after abdom-

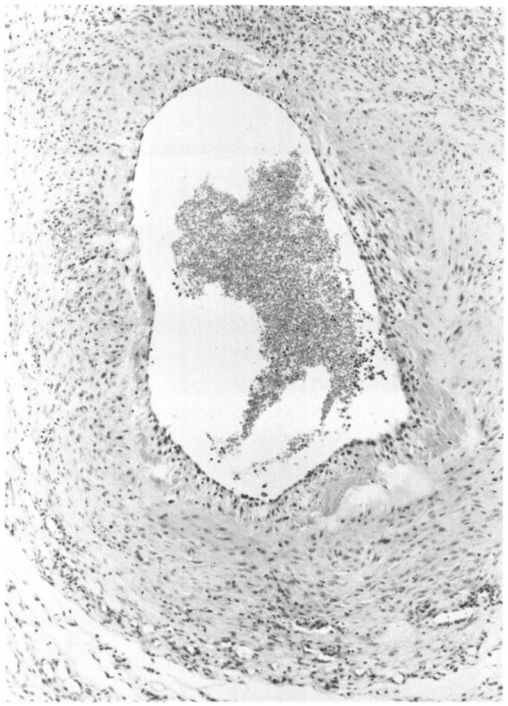

FIG. 5. Old or almost healed lesion of mesenteric artery in the rat in Fig. 3. Arterial wall is replaced by fibromuscular tissue with a slight infiltration of inflammatory cells. H-E stain. ×70.5.

inal X-irradiation. The lesions developed 18 to 36 weeks after the last irradiation and were prominent in the medium-sized arteries. Histologically, the lesions were characterized by fibrinoid necrosis from intima to media with massive infiltration of inflammatory cells including neutrophils and eosinophils. Medial smooth muscles cells usually disappeared and were replaced by granulation tissue. These findings indicate that chronic excess sodium chloride ingestion modified the radiation-induced vascular damage. Although the etiology of periarteritis nodosa is still debated, hypersensitivity, repeated streptococcal infection, drugs, and hypertension have been proposed as causes of this disease (24, 25).

In experimental animals, hypertensive arteritis resembling human periarteritis nodosa has been repeatedly reported by many investigators (18, 26–28). Suzuki *et al.* (26) reported that periarteritis nodosa-like lesions developed and reached 100% at 9 months of age in spontaneously hypertensive rats. As a result of light and electron microscopic observations, they considered that hypertension might induce damage of the internal elastic membrane followed by insudation of plasma proteins into the vascular wall. This plasma insudation might result in the damage of medial cells, especially smooth muscle cells, and induce an inflammatory reaction of the vascular wall. On the other hand, hypertension can be induced by chronic excess sodium chloride ingestion (15–17, 29, 30). Although the precise mechanism of salt-induced hypertension is still unknown, Dahl *et al.* (31) were previously able to develop two distinct strains of rats, one of which always remained normotensive despite a high salt diet (salt resistant), and another which always became hypertensive on a high salt diet (salt sensitive). Thereafter they detected vasopressor substances released from the kidney in the salt-sensitive rats (32, 33). These substances were different from renin and were transferable from salt-sensitive to

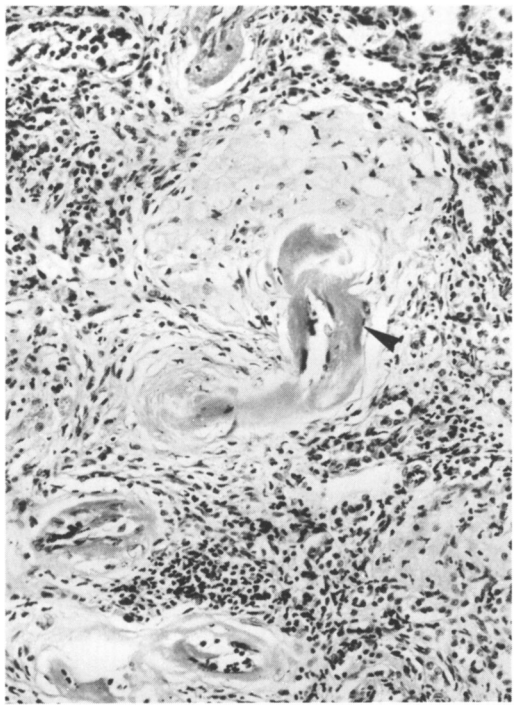

FIG. 6. Renal cortex of the rat in Fig. 2. Glomerular afferent arterioles show subintimal hyaline degeneration (arrow) with glomerular fibrosis. Marked inflammatory infiltration is observed in the interstitium with destruction of renal tubules. H-E stain. ×141.

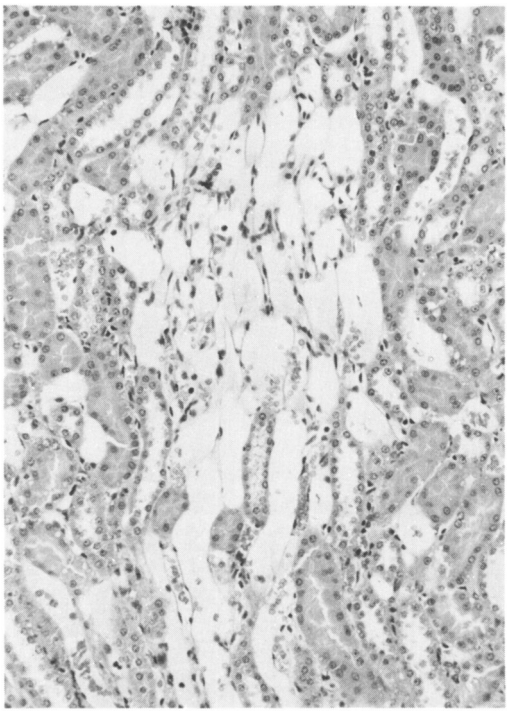

FIG. 7. Renal corticomedullary junction of the rat treated only with sodium chloride. Tubular epitheial cells are degenerated and disappear in small groups of renal tubules. H-E stain. ×141.

salt-resistant rats (34, 35). Moreover, Takeshita and Mark (36) reported that there might be something special about sympathetic nerve stimulation in the salt-sensitive rats. Recently, Tobian (17) suggested that there are antinatriuretic humoral agents produced elsewhere other than kidneys and adrenals.

Unfortunately, we failed to determine the blood pressure, plasma volume, and serum electrolytes of the experimental animals. However, it could be supposed that the animals treated with chronic excess sodium chloride after abdominal X-irradiation were hypertensive, because the kidneys of these animals appeared morphologically quite similar to kidneys of animals with hypertensive nephropathy (37). The kidney was shielded by lead plate at the time of abdominal irradiation. Therefore, the effect of radiation was considered to be negligible for the pathogenesis of these renal lesions. In our present study, on the other hand, it is not known whether the animals treated only with excess sodium chloride were hypertensive or not. However, the kidneys of these animals showed focal degenerative changes of renal tubular epithelium in the corticomedullary junction and medulla without the distinct hypertensive nephropathy observed in the animals with a combination of sodium chloride and X-irradiation. Although the precise mechanism is still unknown, chronic sodium chloride ingestion modifies the radiation-induced vascular injury and produces periarteritis nodosa-like lesions. Therefore, the animals treated with sodium chloride and X-irradiation might be useful models for studying human periarteritis nodosa.

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