

The Effect of Glomerulonephritis on Atherogenesis in Cholesterol-Fed Rats

— A Special Reference to the Glomerular Foam Cell
and the Early Change of Aortic Intima —

Junji OHI

*Department of Pathology, Atomic Disease Institute,
Nagasaki University School of Medicine, Nagasaki, Japan*

Received for publication, June 30, 1987

In order to clarify the effect of glomerulonephritis and hypercholesterolemia in atherogenesis, Masugi nephritis was induced in male Wistar rats fed a high cholesterol diet, containing 2% cholesterol and 0.5% cholic acid in a standard feed. Three groups of rats served as controls; (1) age-matched, untreated normal rats, (2) Masugi nephritis-induced rats, (3) rats fed a high cholesterol diet. The experimental rats showed a high excretion of protein in the urine. Elevation of systolic blood pressure was slight, while a moderate to marked increase of serum lipids and lipoproteins was observed. All experimental rats showed foam cells in the glomerular tufts. An electron microscopic study revealed foam cells in the mesangial matrix and subendothelium of the glomerular capillary. The aorta showed edematous intimal thickening. The experimental rats exhibited the greatest degree of edematous intimal thickening as well as the highest incidence of intimal lesions as compared to the control groups. Ultrastructural observation of the aortic intima revealed a widening of the subendothelial space suggesting early arteriosclerotic change.

These results suggest an additive effect of glomerulonephritis on hypercholesterolemia which promotes the development of aortic intimal lesion in rats.

INTRODUCTION

It has been elucidated clinicopathologically and experimentally that many factors such as hypertension and hyperlipidemia promote atherosclerosis. It is speculated glomerulonephritis accompanied with hypertension and hyperlipidemia promotes atherosclerosis. In this respect, the kidney is one of important organs in studying the

A part of this study was presented at the Winter Meeting of the Japan Atherosclerosis Society, February, 1987.

大井 順二

relationship between organ dysfunction and vascular lesions. Concerning the participation of the kidney in the pathogenesis and development of arteriosclerosis, it is speculated that renal hypertension,¹⁾ non hypertensive renal factor²⁾ and abnormalities³⁾ of blood and electrolytes are important factors of arteriosclerosis.

In glomerulonephritis, it is considered that elevation of blood pressure and hyperlipidemia may play an important role in the pathogenesis and development of arteriosclerosis. However, experimental studies about the additive effect of glomerulonephritis and hypercholesterolemia on arteriosclerosis have not yet been sufficiently done.

The present study was carried out in order to investigate the pathological changes of kidney and aorta in Masugi nephritis-induced rats combined with hypercholesterolemia.

MATERIALS AND METHODS

1. Animals

Male Wistar rats weighing approximately 300gm were randomly divided into four groups as follows; Group I (6 rats) was maintained on stock diet (F₂ food from Funabashi farm) and tap water for 36 weeks; Group II (7 rats) was fed the same diet for 36 weeks and given two intravenous injections of anti-rat-kidney rabbit serum at the 17th and 20th week; Group III (6 rats) was fed a high cholesterol diet of 2% cholesterol and 0.5% cholic acid mixed with stock diet; Group IV (12 rats) was fed the high cholesterol diet for 36 weeks and given two intravenous injections of anti-rat-kidney rabbit serum at the 17th and 20th week.

2. Anti-rat-kidney rabbit serum

Normal rat kidneys were thoroughly perfused with physiological saline through the abdominal aorta. The cortical parts were excised, homogenized and lyophilized. 125mg of rat kidney powder was suspended in physiological saline and injected into the peritoneal cavity of rabbits at the initial injection. The amount of rat kidney injected was increased by 25 mg once a week for 15 weeks, and 500mg was used at the last injection. 3 weeks after the last injection, the rabbits were bled from the carotid artery. The pooled serum was used after inactivation by heating at 56 degrees C for 30 minutes.

3. Blood pressure

Blood pressure was measured every four weeks by the tail cuff method using a RAT automatic blood pressure recorder USM-105-R type. Before measurement, the rats were warmed for 5 to 8 minutes at 35 to 38 degrees C.

4. Urinary protein

Urinary protein was measured by the TCA (trichloro-acetic acid) method using 24 hour-stored urine 1 to 7 days before sacrifice.

5. Sacrifice

All rats were sacrificed at the 37th week. After a 12-hour fast, the blood was obtained from the abdominal aorta under light anesthesia by ethylether.

6. Serum lipids and others

Separated serum was used in the biochemical analysis. The measurements were carried out by the enzyme method for total cholesterol and triglyceride, the heparin-Ca precipitation method for LDL and VLDL, the phosphotungstic acid-Mg precipitation method for HDL-cholesterol, the Biuret method for total protein, and the UV enzyme method for BUN.

7. Light microscopy

After weighing, the excised organs were fixed in 10% formalin. Kidneys were stained with Hematoxyline Eosin, Periodic acid Schiff, Periodic acid Methenamine Silver, Azan-Mallory and Oil red O stain, and then observed by light microscopy. Serial specimens were excised from the ascending aorta to the abdominal aorta in each rat, and then fixed in 10% formalin. These specimens were stained with Hematoxyline Eosin, Elastica van Gieson, Periodic acid Schiff, Alcian Blue and Oil red O stain. 41 specimens from Group I, 73 from Group II, 65 from Group III and 87 from Group IV were observed. The maximum intimal thickness of each specimen was measured with an ocular micrometer.

8. Electron microscopy

Parts of the kidney and aorta were fixed in 1.5% glutaraldehyde, postfixed in 1% OsO₄, dehydrated in ethanol and embedded in epoxy resin. The ultra-thin sections were stained with uranyl acetate and lead citrate and then observed with a JEM-1200EX type electron microscope.

9. Statistics

The resulting data was expressed as mean \pm S. E. and tested by the Student's t-test, and when the data showed a difference of $p < 0.05$, it was judged to be statistically significant.

RESULTS

1. Blood pressure

The transitions of systolic blood pressure are shown in Fig. 1. The systolic blood pressure in Group IV began to increase around the 20th week of the experiment. At the end of the experiment, the systolic blood pressure was 123 ± 2 mmHg, 119 ± 5 mmHg and 119 ± 3 mmHg in Group I, II and III respectively. In Group IV, the systolic pressure was 139 ± 4 mmHg and significantly higher than in the three other Groups (Table 1).

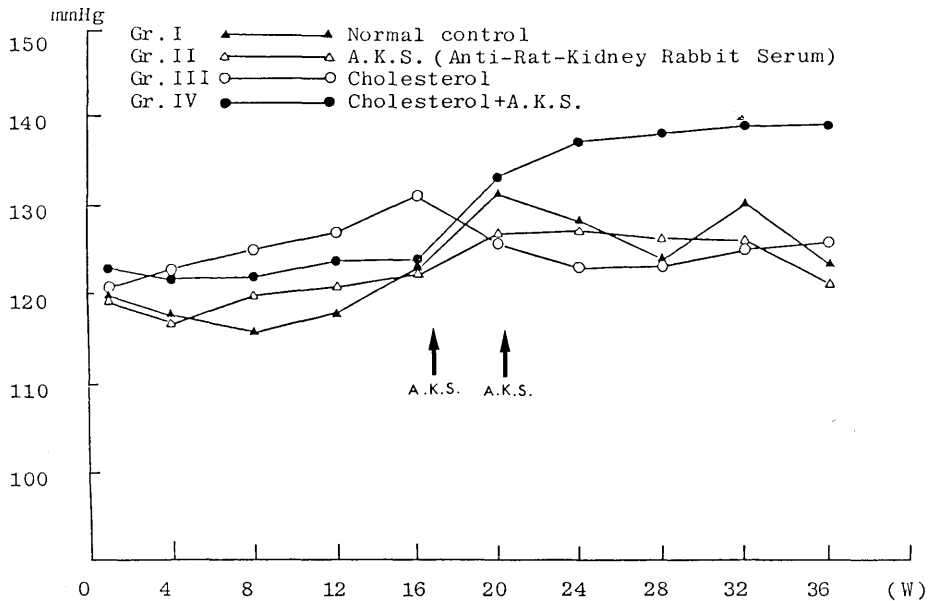


Fig. 1. Blood prssure

Table 1 Systolic blood pressure and urinary protein

Group	n	Systolic blood pressure (mmHg)	Urinary protein (mg/day)
I (CONTROL)	6	123±2	13± 1
II (AKS)	6	119±5	189±27**
III (CHOL)	6	119±3	12± 1
IV (CHOL+AKS)	12	139±4**	233±21***

Values are shown as mean±SE

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ vs. group I

2. Urinary protein

At the end of the experiment, the urinary protein was 13 ± 1 mg/day and 12 ± 1 mg/day in Group I and III respectively. In Group II and IV, the urinary protein was 189 ± 27 mg/day and 233 ± 21 mg/day respectively, and therefore significantly higher than that in Group I and III (Table 1).

3. Organ weight

The body weight in Group II was significantly lower than that in group I. The average organ weight expressed in mg/100gm body weight is shown in Table 2. Weights of the heart in Group III and IV were significantly lower than in Group I. Weights of the kidney and liver in Group II, III and IV were significantly higher than in Group I.

Table 2 Body weight (gm) and organ-body weight ratio (mg/100gm)

Group	n	B.W.	Brain	Heart	Liver	Kidney	Adrenal
I (CONTROL)	6	520±15	430±18	335± 8	2282± 32	549±14	11±0
II (AKS)	7	450±10**	497±13*	346±19	2611± 96*	640±15**	13±0
III (CHOL)	6	531± 8	435± 8	241± 1***	4276±197***	610±18*	13±1
IV (CHOL+AKS)	12	542±22	420±17	292± 4***	4685±237***	632±24*	14±0**

Values are shown as mean±SE.

*: p<0.05, **: p<0.01, ***: p<0.001 vs. group I

4. Serum lipid level and others

Total cholesterol was 390±128 mg/dl and 423±49 mg/dl in Group III and IV respectively, and that in Group IV was significantly higher than those in Group I and II. Triglyceride was 107±11 mg/dl and 178±32 mg/dl in group III and IV respectively, and those levels were significantly higher than those in Group I and II. LDL and VLDL level in Group III and IV were significantly higher than those in Group I and II. Total cholesterol, triglyceride, LDL and VLDL in Group IV were higher than those in Group III, but there was not statistically significant difference between them. HDL-cholesterol was 35±2 mg/dl in Group II, and lower than that in Group I. Group II showed the lowest level of total protein, but there was no significant difference among the groups. No definite tendency was seen in the level of BUN (Table 3).

Table 3 Serum lipids (mg/dl), TP and BUN

Group	n	T-CHOL	TG	LDL	VLDL	HDL-C	TP (g/dl)	BUN (mg/dl)
I	6	73± 5	50± 3	88±11	trace	49±5	6.2±0.1	18±0
II	6	61± 3	61± 3	90±20	trace	35±2*	6.0±0.1	19±1
III	6	390±128	107±11*	254±45*	876±340	46±8	6.6±0.1	18±0
IV	10	423± 49***	178±32**	462±61**	1127±169	66±7	6.3±0.1	19±1

Values are shown as mean±SE

*: p<0.05, **: p<0.01, ***: p<0.001 vs. group I

5. Light microscopic observation of the kidney

In Group II, there was variable thickening of the glomerular basement membrane and mesangium. Scattered glomeruli with capsular adhesions were present. In Group IV, many of the glomeruli were swollen and had foam cells in addition to the alternations seen in Group II (Fig. 2). In the severest instance, more than one-fourth of the glomeruli had foam cells. In Group I and III, no remarkable change of the glomeruli was observed. There was no remarkable change in the tubulus or arteries of any of the groups.

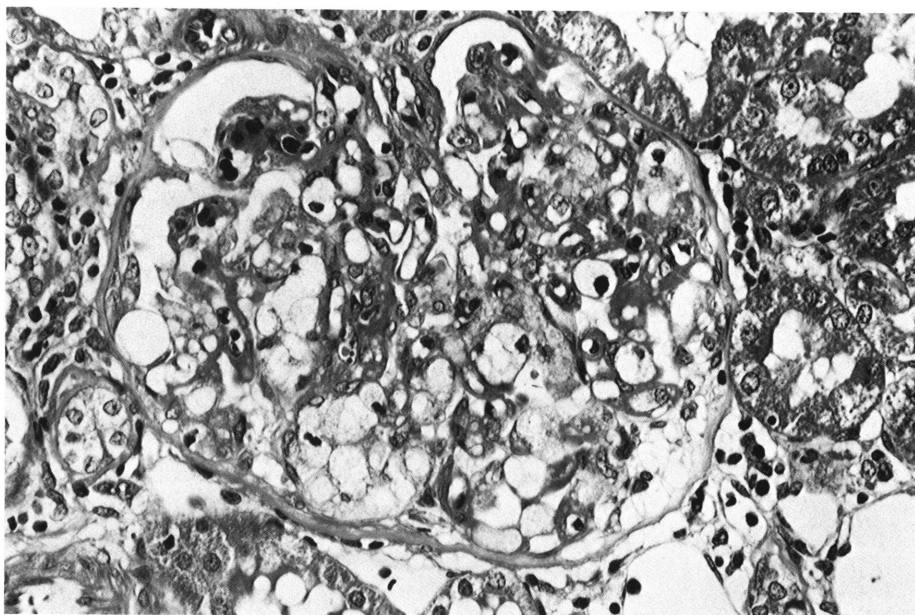


Fig. 2. A kidney section of a rat in group N. The glomerulus is enlarged. Glomerular foam cells and capsular adhesions are seen. (H. E. $\times 380$)

6. Electronmicroscopic observation of the glomeruli

Glomeruli with foam cells in Group N were selected for electron microscopic examination. Numerous electron dense bodies were seen in the epithelium. Fusion of foot processes was also seen in some parts. The glomerular basement membrane showed irregular

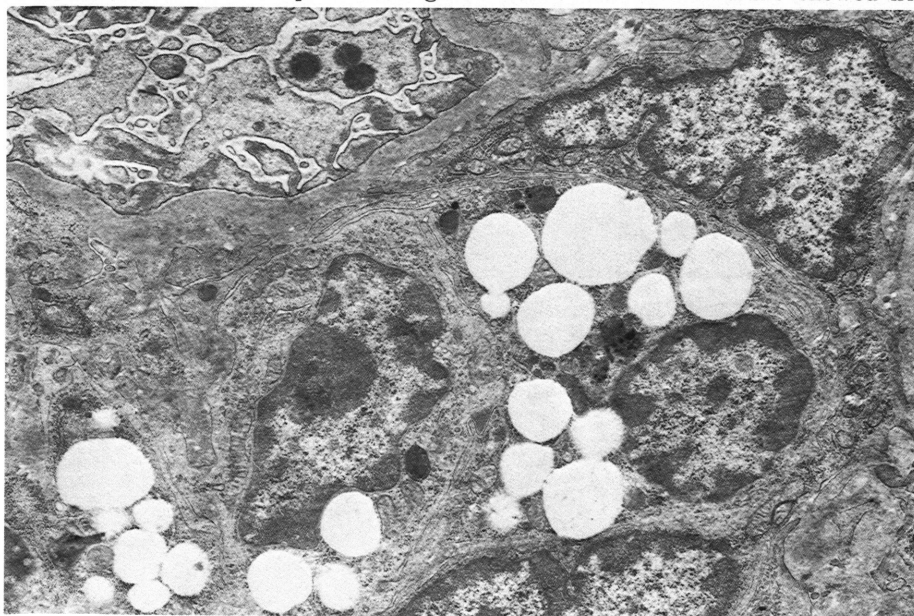


Fig. 3. Foam cells are seen in the mesangium. ($\times 10,200$)

thickening and folding. Mesangial matrix increased, and foam cells were seen mainly in the mesangial area and partially in the subendothelial space (Fig. 3).

7. Light microscopic observation of the aorta

Variable edematous thickenings of the aortic intima were observed (Fig. 4). The incidence of edematous intimal thickening was 54.0%, 18.4%, 4.1% and 2.4% in Group IV, III, II and I respectively. Group IV exhibited the greatest degree of intimal thickening as well as the highest incidence of intimal lesion (Table 4). Histochemically, a part of the edematous intimal thickening was moderately positive for Alcian Blue stain and mildly positive after hyaluronidase digestion, and negative for Oil red O stain (Table 5). In rats of Group IV with marked edematous intimal thickening, intimal cells were observed. Medial smooth muscle cells near the internal elastic lamina showed irregularity in arrangement (Fig. 5).

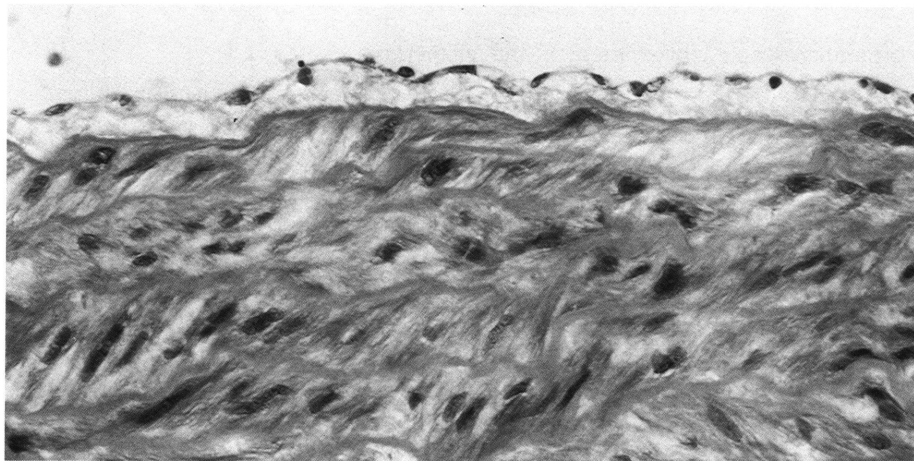


Fig. 4. Mild edematous change of aortic intima of a rat in group IV. (H. E. $\times 380$)

Table 4 Incidence and degree of intimal thickening in the aorta

Group	Incidence of intimal thickening	Intimal thickness (μ)
I (CONTROL)	2.4% (1/41)	0.24 ± 0.24
II (AKS)	4.1% (3/73)	0.30 ± 0.18
III (CHOL)	18.4% (12/65)	$1.46 \pm 0.43^*$
IV (CHOL + AKS)	54.0% (47/87)	$3.79 \pm 0.50^{***}$

Values are shown as mean \pm SE

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ vs. group I

Table 5 Histochemical characteristics of subendothelial edematous area

Stain	Result
PAS	(-)
D/PAS	(-)
AB/(PH2.5)	(++)
AB/(PH1.0)	(++)
TH/AB/(PH2.5)	(+)
TH/AB/(PH1.0)	(+)

PAS (Periodic Acid Schiff stain); D/PAS (PAS after diastase digestion); AB (Alcian Blue stain); TH/AB (AB after human testicular hyaluronidase digestion)

(-)=negative; (+)=mildly positive; (++)=moderately positive

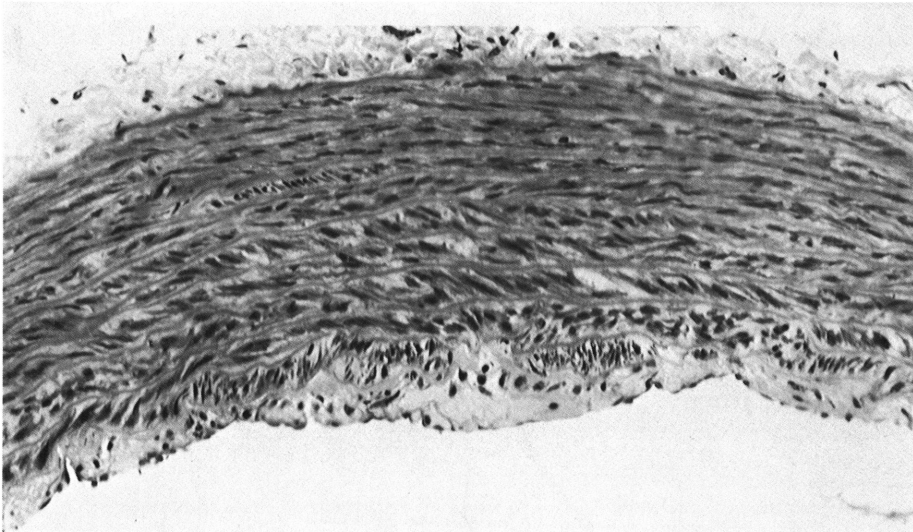


Fig. 5. Marked edematous change of aortic intima of a rat in group IV. (H. E. $\times 190$)

8. Electron microscopic observation of the aortic intima

In the lesions of the aortic intima, the cytoplasm of the endothelial cell showed a mild increase in electron density and projected into the lumen. A large lumen was formed by a cytoplasmic flap and the gap junctions were distended (Fig. 6). The subendothelial space was widened and contained a few collagen fibers and fine granular material within the ground substance (Fig. 7). In rats of Group IV with marked edematous intimal thickening, intimal smooth muscle cells were observed (Fig. 8). These cells were partly surrounded by basement membrane, but myofilament was relatively scarce. Myeline-like structures and relatively abundant mitochondria were present.

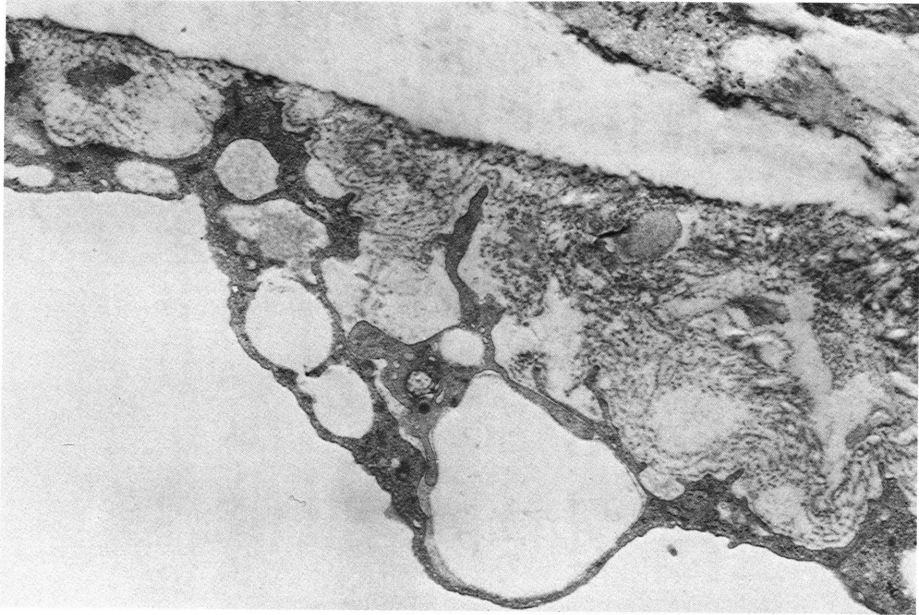


Fig. 6. The gap junction is distended and a large lumen is formed by cytoplasmic flaps. ($\times 8,500$)

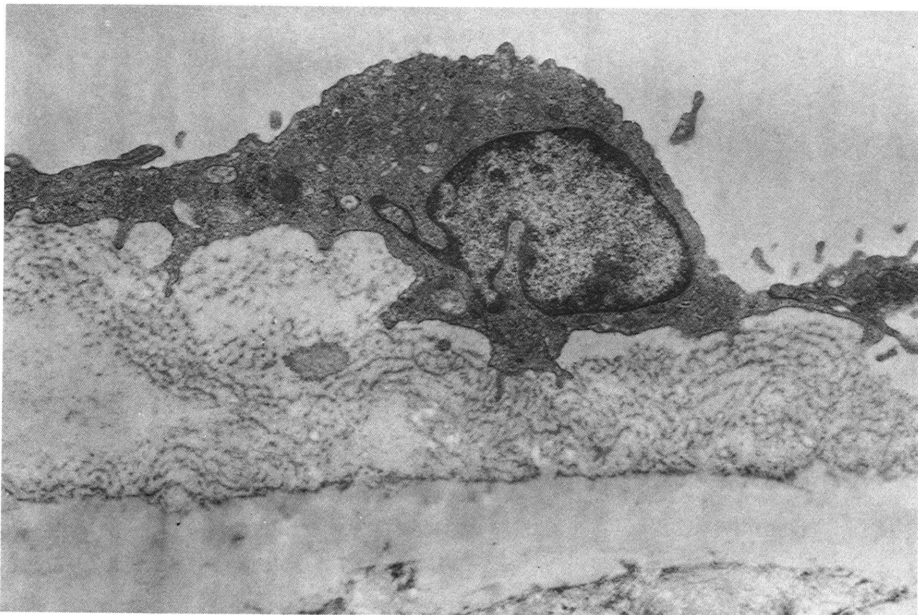


Fig. 7. Mild increase of the subendothelial space. Fine granular material is seen in the subendothelial space. ($\times 10,200$)

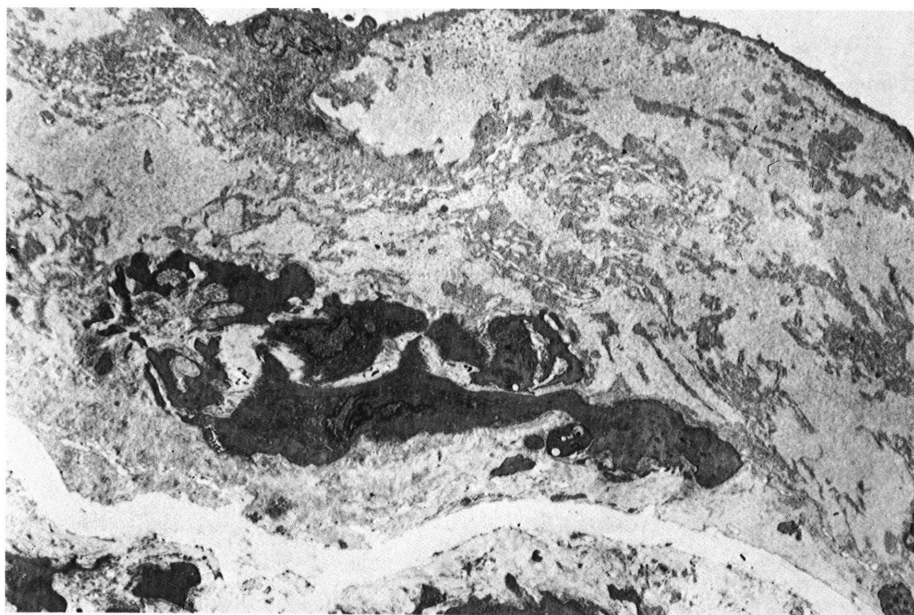


Fig. 8. Marked increase of the subendothelial space.
Intimal smooth muscle cells are seen. ($\times 8,800$)

DISCUSSION

The aorta is an elastic artery which is affected directly by hypertension. It has been elucidated clinicopathologically⁴⁾ that atherosclerotic index in hypertensive cases was higher than in normotensive cases. LIMAS *et al.*⁵⁾ have observed an expansion of the subendothelial space with depositions of acid mucopolysaccharide in the aortic intima of the spontaneously hypertensive rat. In the present study the systolic blood pressure of Group IV was significantly higher than of other groups, but the increase of systolic blood pressure of Group IV was slight. We cannot explain to what extent the increase in systolic blood pressure of Group IV contributed to the development of edematous intimal thickening of the aorta.

Masugi nephritis induced in rats by injections of anti-rat-kidney rabbit serum is an experimental renal disease that resembles the nephrotic syndrome^{6,7)} of infants and children. In the present study two intravenous injections of anti-rat-kidney rabbit serum were performed to maintain and enhance glomerulonephritis. At the end of the experiment an increase of urinary protein was evident in Group II and IV, and therefore the rats were assumed to be in a nephrotic state.

Edematous arterial reaction⁸⁾ and foam cell nest^{9,10,11)} in aortic intima have been observed in hypercholesterolemic rats fed a high cholesterol diet. In the present study, total cholesterol, triglyceride, LDL and VLDL in Group IV were the highest among the

four groups, and Group IV exhibited the highest incidence of edematous intimal thickening. Therefore it is suggested that increased hyperlipidemia by an additive effect of glomerulonephritis promotes the development of aortic intimal lesion. No increase of cholesterol was observed in Group II, which was an unexpected result. VAN LIEW¹²⁾ *et al.* induced chronic serum sickness (CSS) in rats by chronic intravenous immunization with bovine serum albumin, and investigated the relationship between function and histopathology of the kidney. In that experiment, they observed elevation of serum cholesterol level in the rats with moderate to marked CSS, but did not observe it in the rats with mild CSS. However, they did not go into the cause of this phenomenon. Further examination is required in this respect.

KONDO and AKIKUSA¹³⁾ induced Masugi nephritis in rats, and reported capsular adhesion and segmental sclerosis in the glomeruli. In the present study, similar changes were observed. In Group IV, glomerular foam cells were found in addition to the alternations observed in Group II. The presence of foam cells in glomeruli is unusual, and it is known that glomerular foam cells are encountered in conditions associated with nephrotic syndrome. Experimentally, WATANABE¹⁶⁾ *et al.* found glomerular foam cells in rabbits with Masugi nephritis fed a high cholesterol diet and HONDA¹⁷⁾ found them in adrenal regeneration hypertension rats fed a high cholesterol diet. They stated that the foam cells originated from both mesangial cells and circulating monocytes. In the present study, glomerular foam cells were detected only in Group IV. Therefore it is suggested that the appearance of foam cells is dependent upon glomerular damage and hypercholesterolemia.

The tubule epithelium showed no remarkable change, and thus it was considered that there was little effect of non-pressure vasotoxic substance in the kidney.

"Das initiale fettfreie Ödem" of VIRCHOW, gelatinous elevation¹⁸⁾ and edematous reaction⁸⁾ in the aortic intima, which are similar to the aortic intimal change in the present study, have been reported. In the state of "das fettfreie Ödem" or gelatinous elevation, the endothelial cell shows morphological changes and the subendothelial space is occupied with uniform material, the electron density of which is higher than that in edematous reaction. In the state of edematous reaction, there is an expansion of the subendothelial space, but no morphological changes in the endothelial cell. MORIMATSU *et al.*¹⁹⁾ reported the existence of cloudy thickening as an initial change of human atheromatous plaques. Electron microscopically, they revealed extracellular deposits of cellular debris in the same position as that of lipid, and they suggested that irreversible change like cloudy thickening in the aortic intima might depend on the amount of extracellular deposits and the increase of endothelial permeability. In the present study, deposits of lipid were not detected, but an increase of acid mucopolysaccharides was suspected. The changes of the aortic intima observed in the present study were thought to correspond with the state of edematous reaction or gelatinous elevation. Group IV exhibited the highest incidence of these intimal lesions. It

is considered, therefore, that an additive effect of glomerulonephritis on hypercholesterolemia in rats promotes the development of aortic intimal lesions.

YOSHIDA²⁰⁾ *et al.* reported the opening of interendothelial junctions in bifurcation pads at the origin of the internal carotid artery in rabbits prior to the appearance of foam cells in the intima. In the present study, the gap junction were distended, which suggested an increased permeability in the endothelium.

SHIMAMOTO⁸⁾ reported an edematous reaction of the aortic intima in rabbits receiving cholesterol. He described the sticking of platelets and leukocytes to the endothelial surface during the edematous reaction, and mentioned the relationship between this reaction and thrombosis. In the present study, such findings were not detected.

CONCLUSION

1) Hyperlipidemia in rats fed a high cholesterol diet is enhanced by inducing glomerulonephritis

2) Glomerular foam cells are present only in rats fed a high cholesterol diet with glomerulonephritis, and therefore the appearance of glomerular foam cells depends on glomerular damage and hypercholesterolemia.

3) An additive effect of glomerulonephritis on hypercholesterolemia promotes the development of edematous thickening in the aortic intima.

ACKNOWLEDGEMENT

The author wishes to express his sincere gratitude to Emeritus Prof. Issei NISHIMORI, Department of Pathology, Atomic Disease Institute, for his kind guidance in this study and review of this paper. Thanks are also due to Prof. Ichiro SEKINE, Department of Pathology, Atomic Disease Institute, Associate Prof. Masao KISHIKAWA, Department of Pathology, Scientific Data Center of Atomic Bomb Disaster and all the staff members, Department of Pathology, Atomic Disease Institute, Nagasaki University School of Medicine.

REFERENCES

- 1) NISHIMORI, I., MIYAGAWA, N., MORI, M. and TOMITA, S.: Relationship arteriosclerosis and hypertension. *J. Jpn. Atheroscler. Soc.* 2: 2-7, 1974 (Japanese).
- 2) NISHIMORI, I., FUKAZAWA, T. and SHIROMA, S.: Vascular lesions produced by renal cortical extracts. *J. Jpn. Atheroscler. Soc.* 1: 185-191, 1974 (Japanese).
- 3) TODA, T., MIYAZAKI, J., KISHIKAWA, M., TSUDA, N. and NISHIMORI, I.: Relationship between development of arteriosclerosis and kidney; Mesenteric arteriosclerosis, and dialyzed and non-dialyzed kidneys. *J. Jpn. Atheroscler. Soc.* 5: 377-381, 1978 (Japanese).

- 4) SADOSHIMA, S., TANAKA, K., TAKESHITA, M., HIROTA, Y., OMAE, T. and KATSUKI, S.: Atherosclerosis and atherosclerotic disorders in the autopsy cases of Hisayama survey in the recent 10 years. *Fukuoka Acta Med.* **65**: 701–714, 1974 (Japanese).
- 5) LIMAS, C., WESTRUM, B. and LIMAS, C. J.: The evolution of vascular changes in the spontaneously hypertensive rat. *Am. J. Pathol.* **98**: 357–384, 1980.
- 6) HEYMANN, W. and LUND, H. Z.: Lipemic nephrosis in rats. *Science* **108**: 448–449, 1948.
- 7) HEYMANN, W. and LUND, H. Z.: Nephrotic syndrome in rats. *Pediatrics* **7**: 691–706, 1951.
- 8) SHIMAMOTO, T.: The relationship of edematous reaction in arteries to atherosclerosis and thrombosis. *J. Atheroscler. Res.* **3**: 87–102, 1963.
- 9) ROSS, R. and GLOSET, J. A.: The pathogenesis of atherosclerosis. *N. Engl. J. Med.* **295**: 369–377, 1976.
- 10) MAHLEY, R. W.: Atherogenic hyperlipoproteinemia. The cellular and molecular biology of plasma lipoproteins altered by dietary fat and cholesterol. *Med. Clin. North. Am.* **66**: 375–402, 1982.
- 11) JORIS, I., ZAND, T., NUNNARI, F. T. and MAJNO, G.: Studies on the pathogenesis of atherosclerosis. I. Adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. *Am. J. Pathol.* **113**: 341–358, 1983.
- 12) VAN LIEW, J. B., BRENTJENS, J. R. and NOBLE, B.: Relationship of kidney function to immunopathology in chronic serum sickness of rats. *Kidney int.* **24**: 160–169, 1983.
- 13) KONDO, Y. and AKIKUSA, B.: Chronic Masugi nephritis in the rat. An electron microscopic study on evolution and consequences of glomerular capsular adhesions. *Acta. Pathol. Jpn.* **32**: 231–242, 1982.
- 14) MCKENZIE, I. F. C. and KINCAID-SMITH, P.: Foam cells in renal glomerulus. *J. Pathol.* **97**: 151–154, 1982.
- 15) HYMAN, L. R. and BURKHOLDER, P. M.: Focal sclerosing glomerulonephropathy with segmental hyalinosis. A clinicopathological analysis. *Lav. Invest.* **28**: 533–544, 1973.
- 16) WATANABE, T., HATTORI, F. and TANAKA, K.: An experimental study on the origin of foam cells in glomerulonephritis. *Acta Pathol. Jpn.* **32**: 371–383, 1982.
- 17) HONDA, M.: Electron microscopic study on the kidney of adrenal regeneration hypertension rats. A special reference to the glomerular foam cell and nephrotic syndrome. *Acta Med. Nagasaki* **30**: 156–167, 1985.
- 18) HAUST, M. D.: The morphogenesis and fate of potential and early atherosclerotic lesions in man. *Hum. Pathol.* **2**: 1–29, 1971.
- 19) MORIMATSU, M. and NAKASHIMA, T.: Morphologic study of “cloudy thickening” of human aortas as an initial changes of atherosclerosis. *J. Jpn. Atheroscler. Soc.* **2**: 225–237, 1975. (Japanese).
- 20) YOSHIDA, Y., WATANABE, S. and IWATO, T.: Role of naturally occurring intimal thickening at arterial bifurcation in development of diet-induced atherosclerosis. *Yamanashi Med. J.* **1**: 81–92, 1986 (Japanese).