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Stroke-prone Spontaneously Hypertensive Rats as an Experimental Model of Malignant Hypertension

A Pathological Study

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Summary. A light-microscopic study of various organs of stroke-prone spontaneously hypertensive (SHRSP) rats was performed. The rats characteristically developed fibrinoid necrosis of the wall and marked cellular thickening of the intima and media of the arterioles and small arteries of the kidney, testicle, mesentery, adrenal gland, brain, etc. Parenchymal damage of the organs, secondary to the vascular alterations took place. There were no accumulations of lipids in the vascular lesions.

Though stroke has been stressed as a characteristic clinical feature of the SHRSP rats, the cerebral lesions are different from those seen in ordinary cerebrovascular disease. Furthermore, many organs are involved. The overall vascular changes in the brain and other organs are consistent with those seen in malignant hypertension; the SHRSP rat is an excellent model of this disease.

Key words: Stroke-prone spontaneously hypertensive rats – Arteriolar fibrinoid necrosis – Smooth muscle cell proliferation – Malignant hypertension

Introduction

Okamoto and Aoki (1963) established spontaneously hypertensive (SHR) rats through selective successive inbreeding of Wistar Kyoto (WYK) rats. These animals have been regarded as an experimental model of essential hypertension. Okamoto et al. (1974) then separated a colony of stroke-prone spontaneously hypertensive (SHRSP) rats from the SHR rats.

A pathological study of the brain of the SHRSP rats (Ogata et al. 1980 and 1981) revealed lesions consisting predominantly of oedema and tissue degeneration secondary to oedema. There is arteriolar fibrinoid necrosis and occlusion of the lumen. Microinfarcts occur, but larger infarcts are extremely rare. These

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pathological findings differ from typical cerebrovascular lesions, such as intracerebral haemorrhage or infarction which, however, may occur in the SHRSP rat. There is arteriolar fibrinoid necrosis of various organs in the SHRSP rats (Okamoto et al. 1964), which is not always observed in patients with typical cerebrovascular diseases.

This paper describes pathological changes of various organs of the SHRSP rats which developed neurological and bodily symptoms, and discusses which human diseases, the SHRSP rats resemble.

Materials and Methods

The SHRSP rats used were F38-54 generations derived from Prof. Okamoto's laboratory. The rats were fed with stock chow diet (Oriental Company, Tokyo, Japan) containing 0.26% Na, 5.1% fat and 24.0% protein and tap water in air-conditioned rooms under conventional environment with constant temperature (22–23° C) and humidity (50–65%).

Blood pressure and body weight were recorded periodically. Systolic blood pressure was measured without anesthesia by tail-sphygmography.

Twenty-six male and 12 female SHRSP rats, which developed neurological symptoms such as irritability or stupor, with enlarged skull, roughened fur, atrophic testicles in the male, dirt in the pubic area or emaciation lasting for a few days to a few months, were killed by perfusion of the brain with buffered formaldehyde or a mixture of formaldehyde and glutaraldehyde, as described previously (Ogata et al. 1980). Most female rats were breeders.

After gross examination at autopsy, heart, kidney and testicle were weighed before fixation by immersion in 10% formalin. Body weight was recorded to the nearest gram, and organ weight to the nearest $^1/_{100}$ of a gram. Mean value was recorded for paired organs. Organ weight was expressed as organ weight to body weight ratio as a percentage. Lung, large blood vessels in the mediastinum, mesentery, pancreas, adrenal gland and ovary were also fixed in 10% formalin. In 9 rats, the ventricles of the heart were cut into 3 cross sections.

Slices of organs were embedded in paraffin, cut at 4 μm, and stained for light-microscopic examination as described previously (Ogata et al. 1980). Animals showing severe infection, such as pneumonia were excluded from the present investigation.

Six male and 5 virgin female asymptomatic SHRSP rats at 6 months of age were killed to compare organ weight and histological findings between fully grown adults without hypertensive vascular damage and those with advanced vascular changes. Rats killed after developing neurological and bodily symptoms were called symptomatic rats, and those killed at 6 months of age as controls were called asymptomatic rats.

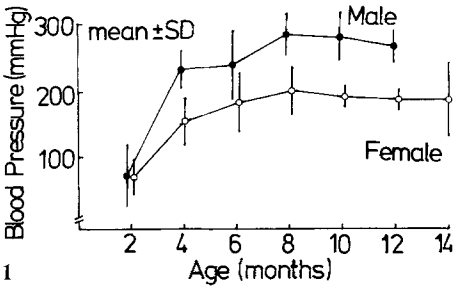
Results

Blood pressure and body weight of male and virgin female rats were recorded in Fig. 1 and 2. Blood pressure of the rats increased before 4 months of age, and remained elevated afterwards (Fig. 1). The average age of the symptomatic rats perfused was 11.1 ± 2.2 (mean $\pm$S.D.) months with a range of 6.0–18.0 months in 26 male, and 16.4 ± 2.7 months with a range of 11.3–20.0 months in 12 female rats.

Pathological Findings

The blood vessels of various organs of the asymptomatic rats did not show hypertensive vascular damage, but the media of the arteries and arterioles appeared thickened.

The ratio of organ weight to body weight is shown in Table 1. The heart



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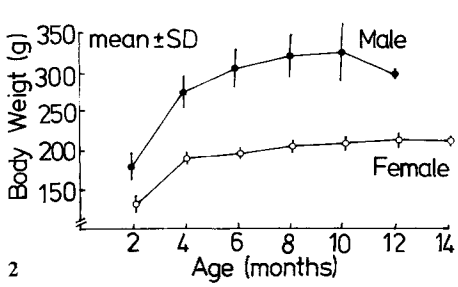

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Fig. 1. Blood pressure (mean ±SD) of SHRSP rats. $N=6$ at each age group

Fig. 2. Body weight (mean ±SD) of SHRSP rats. $N=4$ at each age group

Table 1. Ratio of organ weight to body weight, and body weight (mean ±SD) in symptomatic and 6 months old asymptomatic SHRSP rats

	Male		Female	
	symptomatic (26)	asymptomatic (6)	symptomatic (12)	asymptomatic (5)
Heart	0.61 ± 0.11^{a}	0.42 ± 0.03	0.70 ± 0.09^{a}	0.42 ± 0.02
Kidney	0.63 ± 0.11^{a}	0.43 ± 0.03	0.67 ± 0.05^{a}	0.39 ± 0.01
Testicle	0.34 ± 0.13^{b}	0.52 ± 0.07		
Body weight	238 ± 37^{c}	275 ± 17	177 ± 22	179 ± 9

Values in parentheses indicate number of cases examined.
Statistical difference from asymptomatic rats: ^{a} $p<0.001$, ^{b} $p<0.005$, ^{c} $p<0.05$

and kidney of the symptomatic rats were heavier than those of the asymptomatic rats in both sexes. The symptomatic male rats showed significant reduction of the testicular and body weight compared to that of the asymptomatic rats.

Of 38 symptomatic rats, pleural effusion of serous fluid was observed in 2, and generalized oedema in 1. The arterioles and small arteries of the symptomatic rats showed fibrinoid necrosis and cellular proliferation of the wall with multiplication of the internal elastic lamella in various organs. There was medial thickening in the arterioles and small arteries other than the segments showing vascular lesions. Distinctive pathological findings in each organ of the symptomatic rats are described. The incidence of vascular changes, other than medial thickening, and subsequent alterations of the organs is shown in Table 2.

Heart

There were multiple small myocardial infarcts involving the ventricles in all the cases examined by 3 cross sections (Fig. 5). Perivascular, focal or diffuse fibrosis of the myocardium was present. There was onion-skin like cellular proliferation of the wall of the arterioles causing stenosis or occlusion of the lumen (Fig. 6). Fibrinoid necrosis of the vascular wall was not encountered.

Table 2. Frequency in percentage of vascular and parenchymal lesions of various organs in SHRSP rats with advanced vascular changes

	Male	Female
Cerebral lesions	89 (26)	75 (12)
Myocardial lesions	100 (6)	100 (3)
Nodular lesions of mesentery	84 (25)	92 (12)
Adrenal lesions	53 (19)	70 (10)
Malignant nephrosclerosis	100 (26)	100 (12)
Testicular lesions	100 (26)	

Values in parentheses indicate number of cases examined

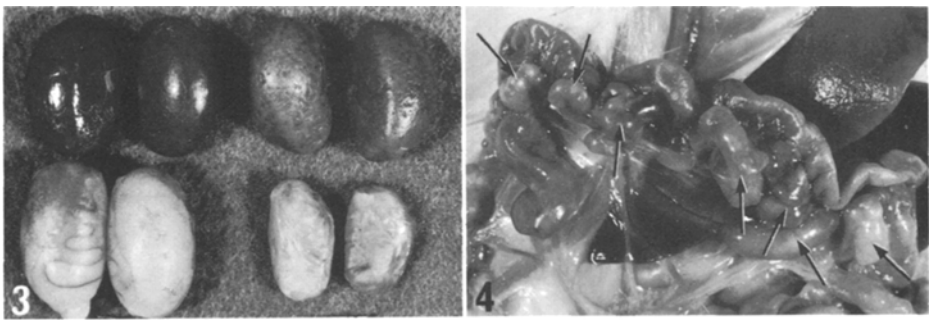

Fig. 3. Finely granular kidneys and atrophic testicles of a 9.0 month-old rat on the right, and normal kidneys and testicles of a 7.0 month-old SHRSP rat as a control on the left

Fig. 4. Nodular lesions (*arrows*) of the mesenteric arteries seen in a 15.0 month-old female rat

Aorta

Subintimal thinning was rarely observed.

Lung

Though rats with severe respiratory infection were excluded, circumscribed small round cell infiltrations around the bronchi were occasionally found. Alveolar and interstitial cells contained haemosiderin in 30 of 36 rats, an incidence of 83%. These cells appeared to be either scattered or in a cluster (Fig. 7). Occasionally, there was congestion, small haemorrhages in the interstitial tissue and oedema fluid in the alveoli. Fibrinoid necrosis of the vascular wall was not encountered.

Mesentery

The mesenteric arteries showed focal or diffuse nodular thickening of the wall and aneurysmal dilatation and thrombosis, which first appeared in the most

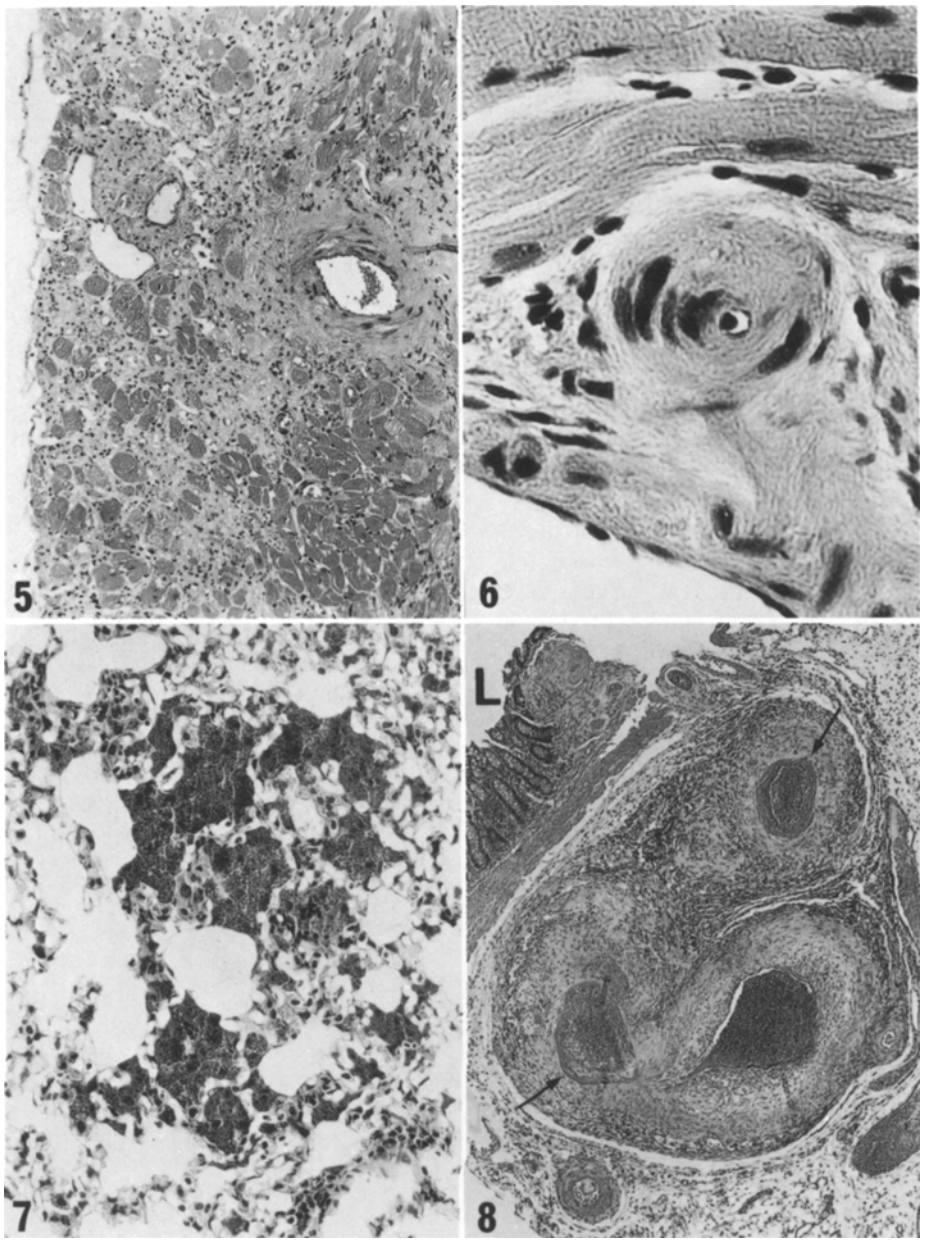

Fig. 5. Small myocardial infarct replaced by connective tissue in the subepicardial region of the left ventricle seen in a 12.8 month-old male rat. HE ×98

Fig. 6. Onion-skin like cellular proliferation of an arteriole in the left ventricle seen in an 11.3 month-old male rat. HE ×540

Fig. 7. Haemosiderin-laden cells in the alveolar lining and interstitial tissue of the lung seen in an 18.7 month-old female rat. HE ×190

Fig. 8. Nodular lesions of the mesenteric arteries within and beneath the intestinal wall seen in an 11.3 month-old female rat. There is enormous thickening of the wall surrounded by cell infiltration. There is subintimal deposition of fibrinoid materials (*arrows*). Intestinal lumen (*L*). HE ×42

peripheral portion of the mesentery (Fig. 4). The nodular lesions consisted of tremenduous cellular and fibrous thickening of the intima and media. Subintimal deposition of fibrinoid materials was present. The fibrinoid materials were granular or homogenous, and stained eosinophilic with HE, red with azocarmine, and blue or purple with PTAH. The vascular changes were often surrounded by small round cell infiltrations (Fig. 8).

Kidney

The kidney appeared somewhat enlarged and firmer than normal. The outer surface was finely granular (Fig. 3). Varying amounts of fibrinoid materials appeared in the capillary tufts of the glomeruli. The afferent arterioles often showed fibrinoid necrosis of the wall. Some glomeruli remained intact among the affected ones. Renal tubules from the obliterated glomeruli contained casts.

The vascular changes, consisting of fibrinoid necrosis of the wall and cellular and fibrous thickening of the intima and media of the arterioles and small arteries, were more advanced in the inner one third of the renal cortex than in the more superficial structures. The vessel wall was often replaced by an onion-skin like cellular proliferation, which led to severe stenosis or thrombotic occlusion of the lumen (Fig. 9).

Adrenal Gland

The adrenal gland was often swollen, and contained blood vessels with fibrinoid necrosis or hyalinosis of the wall and aneurysmal dilatation within the parenchyma and capsule. The hyalinized wall was homogenous or granular, and stained eosinophilic with HE, blue with azocarmine, and tan with PTAH. The gland was often congested, and contained haemorrhages and small infarcts. Collagen balls, organized fibrinoid materials, were occasionally found (Fig. 10).

Testicle

The testicles were small and soft (Fig. 3). The arterioles and small arteries showed cellular and fibrous thickening of the intima and media, aneurysmal dilatation, subintimal deposition of fibrinoid materials, and occlusion of the lumen with thrombus. The seminiferous tubules showed atrophy or disruption of various degrees due to vascular alterations (Fig. 11).

Ovary

The arteries showed only medial thickening.

Brain

Of 38 symptomatic rats, 31 showed cerebral lesions. Lesions, consisting of rarefaction of the neuropil with preservation of the neurons were found in the neocortex and subjacent white matter in 29. These lesions appeared in the para-sagittal region of the parietal lobes in 29, and in the frontal lobes in 7. Arteriolar fibrinoid necrosis was found in the neocortex in 29, thalamus

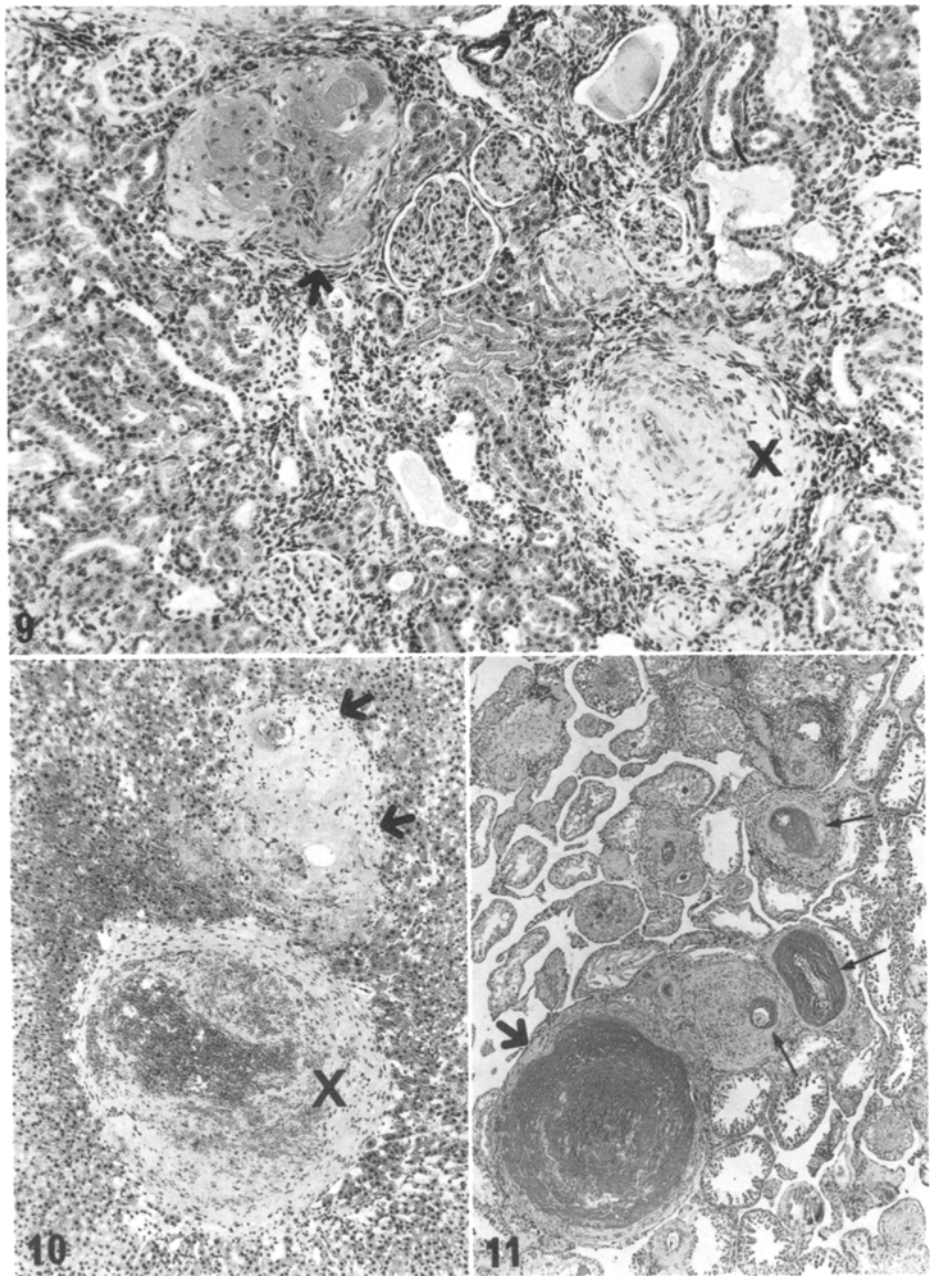

Fig. 9. Renal lesion seen in an 11.3 month-old female rat. There is necrotizing glomerulitis with the afferent arteriole showing fibrinoid necrosis of the wall (*arrow*). There is proliferative endoarteritis (*X*). Intact glomeruli are seen next to the obliterated glomeruli. HE ×126

Fig. 10. Adrenal gland of a 16.0 month-old female rat. There is fibrinoid material (*arrows*) around the small blood vessels and a fibrin ball containing thrombus (*X*). HE ×74

Fig. 11. Atrophic testicle of an 11.0 month-old male rat. The arterioles show enormous thickening of the wall, subintimal deposition of fibrinoid materials (*small arrows*), and aneurysmal dilatation of the lumen and thrombosis (*large arrow*). The seminiferous tubules are disrupted. HE ×40

in 3, nucleus caudatus putamen in 2, and vermis of the cerebellum in 7. One case showed lesions consisting of arteriolar fibrinoid necrosis and petechial haemorrhages only in the vermis of the cerebellum. There were massive intracerebral haemorrhages in 2 in the cortex of the parietal lobe, 1 measuring 300 μm and another measuring 900 μm. Multiple petechial haemorrhages were seen in 22. Multiple old haemorrhages, consisting of small collections of haemosiderin-laden macrophages, were seen in 13. A large old cystic haemorrhage measuring 4 mm in diameter was present in the nucleus caudatus putamen in 1. Microinfarcts were found in the neocortex in 3. Large infarts were not encountered. The major arteries at the base of the brain showed medial thickening only.

Discussion

The SHRSP rats are very susceptible to respiratory infections, because of a decrease in cellular and humoral immunity (Takeichi et al. 1980). Thus they may die of respiratory infections before developing the characteristic hypertensive vascular changes. For this reason the present investigation was carried out by killing the symptomatic animals by perfusion in order to observe advanced hypertensive vascular and parenchymal changes, not modified by superimposed infection.

In the SHRSP rats, there is a marked rise in blood pressure which develops early in life, and is sustained throughout life. The heart gains weight in relation to the severity and duration of hypertension.

The arterioles and small arteries of various organs, such as kidney, mesentery, testicle, brain, etc, show subintimal deposition of fibrinoid materials and cellular and fibrous thickening of the wall. These changes involve only the distal ramifications and small muscular arteries and leave larger muscular arteries and elastic arteries unaffected. The cellular and fibrous thickening of the wall bear some resemblance to human atheromatous plaques, but there are no accumulations of lipids.

Cellular proliferation of the intima and media of the arterioles and small arteries is consistent with the proliferative endoarteritis seen in malignant nephrosclerosis in humans (Wilson and Byrom 1936). Furthermore, cellular proliferation in vascular lesions in various organs of the SHRSP rats exceeds that seen in humans. Clowes and Clowes (1980) reported more severe myointimal thickening in experimentally de-endothelialized aorta and carotid arteries in SHR rats in comparison with that in WYK rats. This change could be controlled with antihypertensive medication. Yamori et al. (1981) reported a greater growth rate of cultured aortic smooth muscle cells from the SHRSP rats in comparison with those from the normotensive WYK rats. Therefore, it is assumed that sustained severe hypertension induces tremendous cellular proliferation in the vascular wall in response to hypertensive vascular injury, in which the genetic characteristics of the smooth muscle cells of the SHRSP rats may play a role.

The pathological changes in the kidney of the SHRSP rats are characterized by necrotizing glomerulitis, fibrinoid necrosis of the wall of the arterioles and small arteries, and proliferative endoarteritis. These histological changes appear to be identical with the lesions associated with human malignant hypertension

(Wilson and Byrom 1963). Though there were many obliterated glomeruli, a significant number of normal glomeruli remained intact, even in the rats with highly advanced hypertensive vascular changes. Plasma creatinine concentration was reported to be 0.64 ± 0.14 mg/dl in the SHRSP rats of 65 weeks of age, with a normal value of 0.40 ± 0.07 mg/dl in the WYK rats of 8–65 weeks of age (Feld et al. 1977). Serum blood urea nitrogen concentration was 26.5 ± 2.8 mg/dl in 18 month-old SHRSP rats and 16.7 ± 1.3 mg/dl in 18 month-old WYK rats (Ohtaka et al. 1980). Though creatinine and BUN values are significantly higher in SHRSP rats than in WYK rats, these values indicate that the SHRSP rats are unlikely to die of uraemia.

In assessing the cause of death it is evident that some rats have obvious causes, such as brain death or massive peritoneal or retroperitoneal haemorrhage (Ogata et al. 1980). However, many animals do not possess such an obvious cause. Death in such animals is assumed to occur as a result of generalized severe hypertensive vascular change and subsequent parenchymal damage to the organs, in which intercurrent infections often play a significant role. The pulmonary haemosiderin-laden cells in association with generalized oedema, pleural effusion and oedema fluid in the alveoli, may be the pathological counterpart of congestive heart failure, which could be the cause of death.

The syndrome of malignant hypertension has three chief components: albuminuric retinitis, rapidly progressive renal failure, and the presence after death of arteriolar necrosis (Pickering 1962). Encephalopathy frequently occurs in the course of malignant hypertension. The brain of patients with hypertensive encephalopathy shows arteriolar fibrinoid necrosis, microinfarcts and petechial haemorrhages (Chester et al. 1978). Many investigators have demonstrated cerebral oedema in hypertensive encephalopathy (Adachi et al. 1966). The patients may die of intracerebral haemorrhage, heart failure or renal failure.

The SHRSP rats show a marked and sustained rise in blood pressure from an early period in life. They develop fibrinoid necrosis and proliferative changes in the wall of the resistance arteries and subsequent parenchymal damage in various organs. There is no accumulations of lipid in the vascular lesions. The cerebral lesions consist of arteriolar fibrinoid necrosis, microinfarcts, petechial haemorrhages and oedema. The eyes are reported to develop hypertensive neuroretinopathy (Irinoda 1977). The overall vascular changes of the rats in respects to the nature and distribution are consistent with those seen in malignant hypertension in humans (Wilson and Byrom 1936).

The terminology, stroke, has been emphasized as a characteristic feature since the establishment of this particular strain of SHR rats. However, the cerebral lesions are different from those of the ordinary cerebrovascular diseases, and other vital organs are simultaneously involved with severe hypertensive vascular changes. Therefore, it is evident that the clinicopathological features of the SHRSP rats resemble those of human malignant hypertension rather than those of ordinary stroke. The SHRSP rats should not be regarded as an experimental model of cerebro-vascular accident in the benign phase of hypertension in man.

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