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Relationship between blood pressure level, renal histopathological lesions and plasma renin activity in fawn-hooded rats

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Summary. The fawn-hooded rat (FH rat) develops hypertension accompanied with focal and segmental glomerulosclerosis and proteinuria, resulting in premature death. In a first experiment the relationship between renal lesions and blood pressure at various ages was investigated. In a second experiment blood pressure was measured weekly from 10 to 38 weeks of age in a number of male FH rats, followed by examination of renal tissues at 40 weeks of age. Plasma renin activity (PRA) had also been determined in individual FH rats. FH rats aged 4.5 weeks had no renal morphological abnormalities. The severity of the glomerulosclerosis increased with age and showed a positive relationship with blood pressure. The scores of the proteinaceous tubular casts also increased with age and they, too, showed a positive correlation with blood pressure. The severity of glomerulosclerosis and proteinaceous casts at 40 weeks of age was related positively to the course of blood pressure throughout life. The final blood pressure level showed a positive correlation with final PRA values. Only FH rats with malignant nephrosclerosis had high PRA values. The renal glomerular and vascular lesions in the FH rat, most likely caused by the hypertension, progressively deteriorate to malignant nephrosclerosis. At that stage PRA values are increased and may be contributing to the development of renal vascular lesions and acceleration of the hypertension.

Keywords: fawn-hooded rat, hypertension, glomerulosclerosis, plasma renin activity, malignant nephrosclerosis, vascular lesions

The fawn-hooded rat (FH rat) develops focal and segmental glomerulosclerosis accompanied with proteinuria and hypertension, progressively deteriorating to malignant nephrosclerosis and premature death. Previous pathological examinations of FH rat kidneys at various ages by electron microscopy and immune fluorescence (Kuijpers & Gruys 1984) demonstrated that the glomerular changes were not due to immune complex disease; it was concluded that the glomerulosclerosis as well as the proteinuria are consequences of the hypertension. Moreover, plasma renin activities (PRAs) were normal in young rats and tended to decline with age (Kuijpers & de Jong 1982).

The present study consisted of two experiments. The first experiment investigated the

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severity of the glomerulosclerosis and proteinaceous tubular casts at various ages and their relationship to the blood pressure level at those ages. However, the severity of the glomerulosclerosis is expected to be influenced not only by the degree of hypertension, but also by the duration of the increased blood pressure. The second experiment was therefore focused on the relationship between blood pressure throughout life and the renal sequelae. At the termination of this experiment PRA was assayed in individual FH rats as renin may contribute significantly to the glomerular and vascular lesions observed in malignant nephrosclerosis (Chatelain *et al.* 1980; Helmchen & Kneissl 1981).

Materials and methods

Experiment 1. The blood pressure of male FH rats, fed a commercial diet, aged 4.5, 19, 27 and 38 weeks ($n=4\times10$), and 48 weeks, respectively, ($n=5$) was measured by the direct technique. This technique involves surgery under ether anaesthesia for insertion of a cannula in the carotid artery early in the morning and blood pressure measurement after complete recovery from anaesthesia at the end of the day.

Experiment 2. In 32 male FH rats, which were fed a purified diet, the systolic blood pressure was measured weekly from the age of 10 weeks onwards by using the tail cuff method (Leenen & de Jong 1971; Kuijpers *et al.* 1986). In addition the animals were weighed weekly. The individual courses of systolic blood pressure throughout life were calculated on the basis of the areas bounded by the individual curves and the x axis, 0 mmHg being the lower limit. The relationship between final systolic blood pressure level and PRA was studied by using the average individual blood pressure readings from 30 to 38 weeks of age.

At 40 weeks of age, the rats were decapitated. Blood from the trunk was collected in polyethylene centrifuge tubes containing 0.2 ml of a 5% w/v EDTA solution in isotonic saline and subsequently placed in ice. The plasma was centrifuged at -4°C, and stored at -20°C. PRA was measured as described before (Ten Berg & de Jong 1980).

Renal histopathology. In both experiments, after decapitation of the animals the kidneys were removed and subsequently preserved in Dubosq-Brazil solution. Sections of 2–4 μm were stained with tetra-Periodic Acid-Schiff (tetra-PAS), Periodic Acid Methenamine silver–Martius scarlet and blue (PAMS-MSB), Harris' haematoxylin and azophloxin (HA), and Masson's trichrome stain. The histopathological changes of glomerules, tubules and vasculature were evaluated separately on coded slides.

For glomerular evaluation, 50 glomeruli per kidney section were observed, and checked for the presence of moderate (*A*), severe segmental glomerulosclerosis (*B*) or global glomerulosclerosis or atrophy (*C*). The altered glomeruli were counted, so *A*, *B* and *C* reflect the incidence of the various glomerular lesions per 50 glomeruli. The overall glomerulosclerosis score was calculated as

$$\frac{A+2B+3C}{100}\ \text{(GS-score)}.$$

The proteinaceous tubular casts were quantified using scores ranging from 0 to 5+ (PTC-scores): 0, no casts; 1+, very few casts; 2+, a few casts; 3+, moderate quantities of casts; 4+, great quantities of casts; 5+, extensive quantities of casts, as seen in end-stage kidneys. Proteinaceous tubular casts reflect the severity of the proteinuria: in previous studies (Kuijpers *et al.* 1986) it was noted that animals with heavy proteinuria (>100 mg per day) had renal tubular cast scores of 4+ and 5+. The morphological characteristics of the glomerular and tubular changes as well as the interstitial changes have been described in detail earlier (Kuijpers & Gruys 1984).

The renal arteries and arterioles were examined for alterations with emphasis on those associated with hypertension. The

renal vascular lesions were classified according to Robertson (1968).

'Benign' lesions refer to those vascular changes that are purportedly associated with benign hypertension, namely hyalin and hyperplastic arterio(lo)sclerosis. In our study hyalinization of arteries and arterioles was characterized by fading of the tunica media, visible as acellular, thickened areas in the vascular wall. It was distinguished from fibrinoid using Masson's trichrome-stained sections. Fibrinoid stained red with Masson's trichrome and was strongly eosinophilic with HA. Hyalinized areas stained faintly green with Masson's trichrome and were faintly eosinophilic with HA. Hyperplastic arterio(lo)sclerosis was characterized by medial smooth muscle cell hyperplasia.

'Malignant' lesions refer to those vascular changes that are ascribed to the presence of malignant hypertension (Robertson 1968; MacMahon 1968; Jones 1974; Chatelain *et al.* 1980) comprising arterial necrosis and arteritis, fibrinoid deposits and insudation of blood components in the arterial wall.

Intimal proliferation and widening of the subendothelial space are early lesions in hypertension (Limas *et al.* 1980), and may be encountered in benign as well as malignant hypertension.

Results

Renal histopathology and relationship with blood pressure

Experiment 1. Animals aged 4.5 weeks did not show any evident renal pathological changes. A few animals had slightly dilated distal and collecting tubules. From the age of 19 weeks onwards, focal glomerulosclerosis as well as tubular changes were found in each rat. Interstitial mononuclear cells were minimal at 19 weeks but they increased with age. The severity of the glomerulosclerosis increased with age and showed a positive relationship with blood pressure: animals with high blood pressure values had a more severe degree of glomerulosclerosis ($r=0.41$, $P<0.05$; Fig. 1).

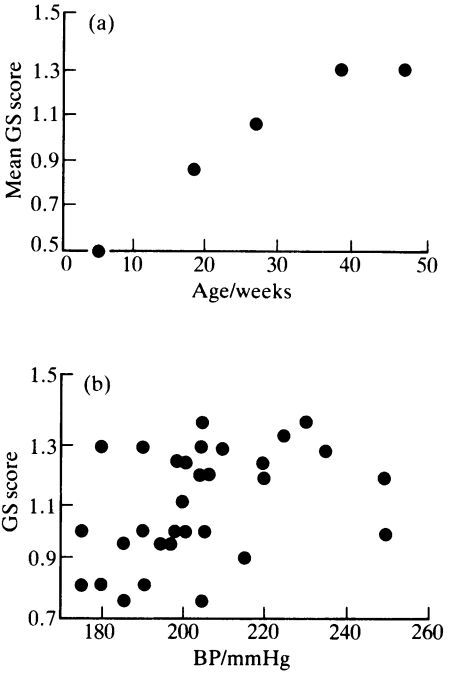

Fig. 1. Overall glomerulosclerosis (GS) scores: *a*, at various ages and *b*, as a function of individual mean blood pressure (BP) (*b*, $r=0.41$, $P<0.05$).

The proteinaceous tubular cast scores also increased with age and showed a positive relationship with blood pressure ($r=0.59$, $P<0.01$; Fig. 2). The relationship between glomerulosclerosis and proteinaceous tubular casts was characterized by $r=0.62$.

In animals aged 4.5–38 weeks, no distinct renal vascular changes could be diagnosed except for an occasional arterial medial thickening. In more severely affected kidneys, slight hyalinization was seen occasionally.

The 48-week-old FH rats all showed hyalin and hyperplastic arterio(lo)sclerosis.

Experiment 2. FH rats in the second experiment, killed at the age of 40 weeks showed a variable degree of glomerulosclerosis and proteinaceous tubular casts. The glomerules in animals with malignant nephrosclerosis showed fibrinoid deposition and

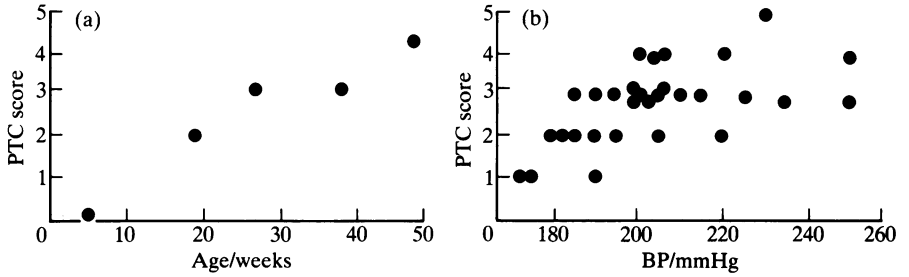

Fig. 2. Proteinaceous tubular casts (PTC) scores: *a*, of various ages and *b*, as a function of the individual mean blood pressure (BP) (*b*; $r=0.59$, $P<0.01$).

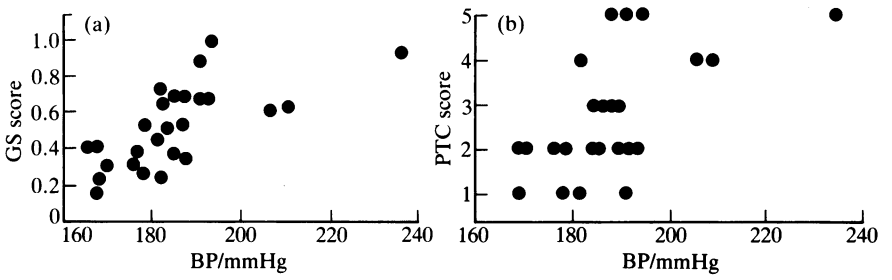

Fig. 3. Individudal average systolic blood pressure from 10 to 38 weeks of age (BP) in relation to: *a*, individudal glomerulosclerosis (GS) scores at 40 weeks of age (*a*, $r=0.65$, $P<0.01$) and *b*, individual proteinaceous tubular casts (PTC) scores at 40 weeks of age (*b*; $r=0.63$, $P<0.01$).

haemorrhages, or ischaemic changes and necrosis, and a number of the glomerules were atrophic.

The positive relationship between renal lesions at 40 weeks of age and blood pressure from 10–38 weeks of age was characterized by $r=0.65$ for glomerulosclerosis and $r=0.63$ for tubular casts ($P<0.01$, Fig. 3). The correlation coefficient of glomerulosclerosis and tubular casts at 40 weeks of age was $r=0.83$ ($P<0.01$). The glomerulosclerosis scores and PRA values also showed a positive relationship ($r=0.49$, $P<0.01$).

The vascular lesions found in individual FH rats of the second experiment have been summarized in Table 1. Hyperplastic arterio-(lo)sclerosis was present in the majority of the animals, sometimes accompanied with hyalinization. Arterio(lo)sclerosis occurred even in animals with minor glomerular and/or tubular abnormalities, summarized as 'nephrosis' in Table 1. Intimal proliferation was found in renal arteries, irrespective of other benign or malignant changes such as focal necrosis and single cell necrosis.

Necrosis was found mainly in subendothelial areas of the renal arteries (Fig. 4) and occasionally in arcuate and interlobular arteries of end-stage kidneys. Arterial necrosis was only seldom accompanied with perivascular inflammation and/or fibrinoid deposition. In a few cases, single cell necrosis with karyorrhexis of subendothelial smooth muscle cells was observed. Near areas of subendothelial necrosis or single cell necrosis adherence, and sometimes infiltration, of luminal monocytes was noted (Fig. 4). Focal necrosis or single cell necrosis occurred in kidneys otherwise relatively unaffected (nephrosis 1+ to 3+). Overt necrotizing arteritis occurred only in malignant nephrosclerosis.

Table 1. Qualification of vascular lesions in individual FH rats in relation to plasma renin activity (PRA, ng angiotensin per ml per hour) and average blood pressure (mmHg)

Animal number	PRA	Average blood pressure		Nephrosis	Qualification of renal vascular lesions		
		10–38 weeks	30–38 weeks		benign	malignant	other
1	12.67	190	192	3+	hl		i
2	10.77	184	196	3+	hl	n	i
3	died at 3 months of age						
4	9.26	186	191	0	hp,hl		i
5	12.65	178	177	1+	hl		i
6	15.32	187	193	1+	hl		
7	11.44	171	172	2+	hp,hl		
8	14.75	191	199	1+	hl	n	i
9	9.93	188	199	3+			i
10	24.37	197	217	4+	hp,hl	n,f	
11	26.79	191	202	2+	hl	n	i
12	8.33	173	178	1+	hl		i
13	3.56	172	180	1+	hp,hl		
14	40.72	209	229	4+	hp,hl	n,f	
15	31.17	212	237	4+	hp,hl	n,f	
16	16.85	181	191	2+	hl		i
17	7.15	195	210	1+	hl		i
18	4.42	182	191	1+	hl		
19	11.02	175	185	1+	hp,hl		i
20	11.19	171	172	3+	hp,hl		
21	13.58	185	197	2+	hl		
22	7.34	192	208	3+	hl		
23*	14.25	220	238	5+	hp,hl	n,f	i
24	14.52	188	205	3+	hp,hl		i
25	24.06	240	266	5+	hp,hl	n,f	i
26	18.30	190	208	4+	hl		
27	12.27	195	211	2+	hl		
28	9.04	182	186	1+	hl		
29	9.03	195	208	4+	hp,hl	n	i
30	18.27	196	203	2+	hl		i
31*	36.32	245	261	5+	hp,hl	n,f	i
32	21.57	—	—	1+	hl		

* Killed at 38 weeks of age in extremis.
Symbols used for vascular lesions:

hp Hyperplastic arterio(lo)sclerosis.
hl Hyalin arterio(lo)sclerosis.
n Arterial necrosis.
f Arterial fibrinoid deposits.
i Intimal proliferation.

Deposition of fibrinoid in hyalinized hyperplastic arteries and arterioles was seen in end-stage kidneys, often accompanied with insudation of erythrocytes in vessel walls. The kidneys showing these features of malignant nephrosclerosis had been taken from those animals that were killed at 38 weeks of age in extremis (animals 23 and 31), and from a few animals killed at the termination of the experiment (animals 10, 14, 15 and

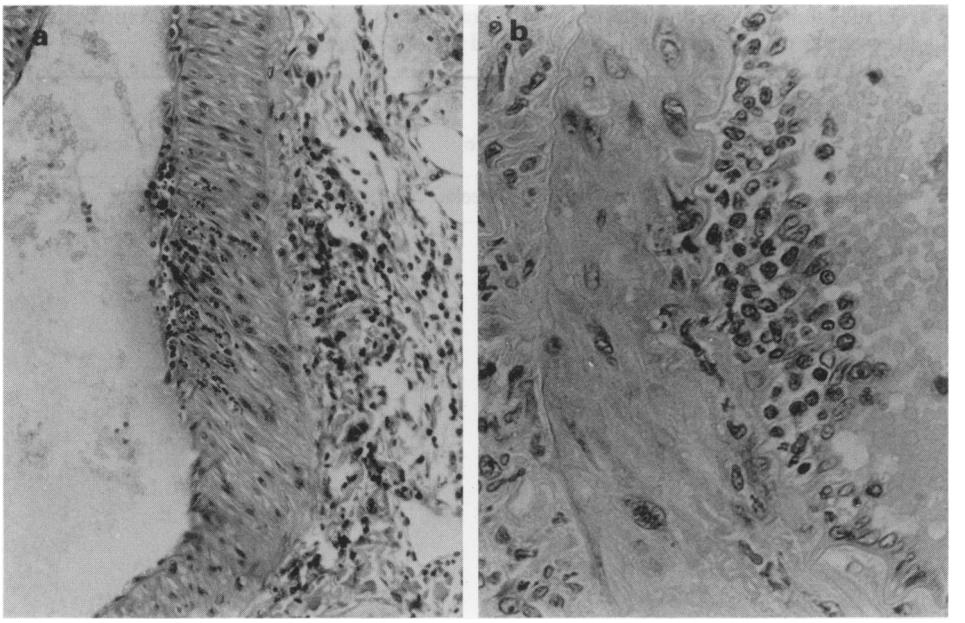

Fig. 4. Focal subendothelial necrosis in a renal artery of a 40-week-old male FH rat. *a*, Masson's trichrome, × 200. *b*, Adherence of luminal monocytes near area with single cell necrosis in a 40-week-old male FH rat. H & A, × 300.

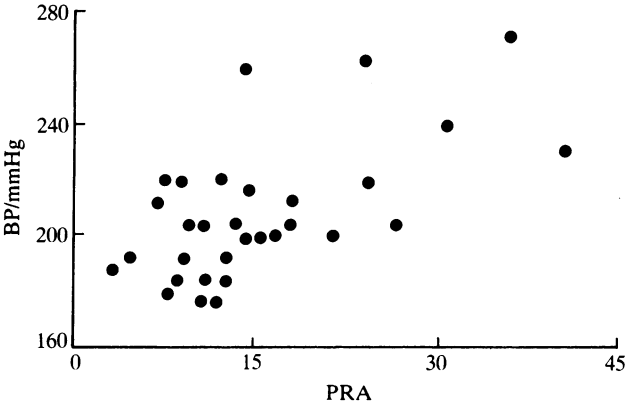

Fig. 5. Blood pressure level (BP) and plasma renin activity (PRA, ng angiotensin/ml/h) in 40-week-old male FH rats ($r=0.68$, $P<0.001$).

25). The latter animals were losing weight and showed clinical manifestations of deterioration.

Plasma renin activity and blood pressure

The final blood pressure level of individual FH rats showed a positive relationship with PRA values at 40 weeks of age ($r=0.68$, $P<0.001$, Fig. 5). Only rats with malignant nephrosclerosis had very high PRA values (Table 1). The glomerulosclerosis scores and PRA values also showed a positive relationship ($r=0.49$, $P<0.01$).

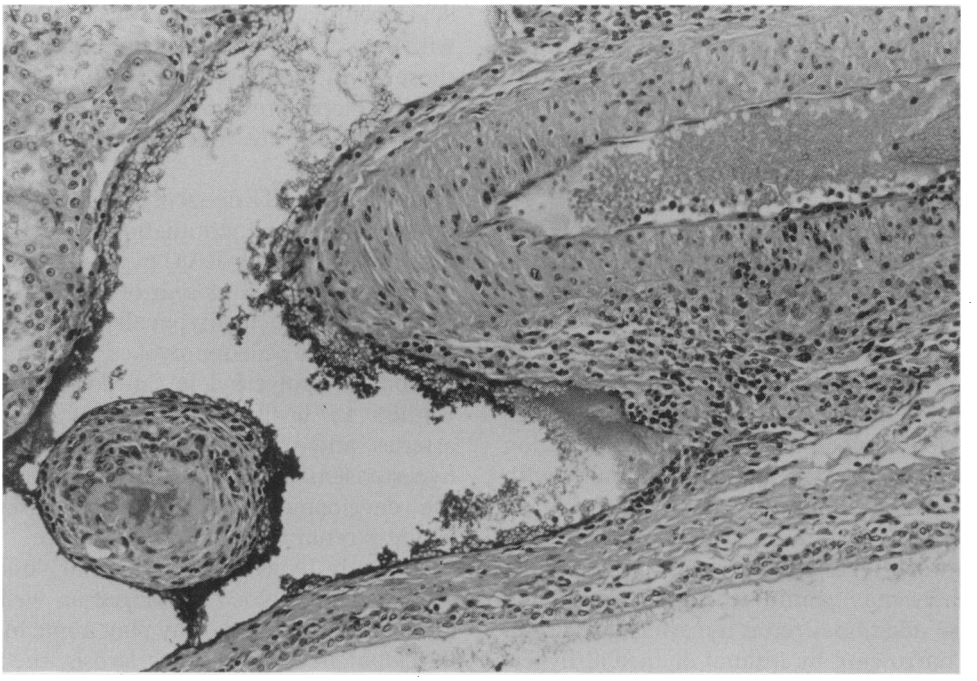

Fig. 6. Focal necrosis in renal artery and endarteritis obliterans of renal artery branch in a 40-week old male FH rat. Masson's trichrome, × 150.

PRA values of the FH rats of Experiment 2 were higher than those found previously in FH rats at various ages (Kuijpers & de Jong 1982). This may reflect the low sodium content (0.06% w/w) of the purified diet used in Experiment 2, as compared to the 0.24% w/w sodium of the commercial diet used in the previous studies.

Discussion

The present study illustrates that the intensity of the renal lesions in FH rats is related to the severity of the hypertension in individual animals. The relationship between renal glomerular lesions and tubular casts and the blood pressure course throughout life is slightly more pronounced than with terminal values. Mandal (1979) reported similar correlations between renal histopathology and blood pressure for spontaneously hypertensive rats (SHR). The above findings support the assumption that the glomerulosclerosis and proteinuria in the FH rat are induced by existing hypertension. The possibility that renal glomerulosclerosis is the underlying cause of the raised blood pressure cannot be ruled out, but previous studies have revealed that the glomerulosclerosis in the FH rat is not of immune complex origin (Kreisberg & Karnovsky 1978; Kuijpers & Gruys 1984); furthermore the PRA values were normal in young FH rats (Kuijpers & de Jong 1982).

The renal vascular lesions in FH rats evolve similarly to those in SHR, beginning with medial thickening through smooth muscle cell hyperplasia, followed by hyalinization in kidneys with severely affected parenchyma (Limas *et al.* 1980). However, even in 48-week-old SHR, malignant hypertensive lesions were not encountered (Limas *et al.* 1980) whereas in FH rats at 40 weeks of age, increasing numbers of animals developed malignant nephrosclerosis.

Malignant arterial lesions in 40-week-old animals—except for a few exceptions—were associated with high final blood pressure levels and high PRA values (Table 1). These observations suggest the development of an accelerated 'renal' phase in the hypertension of FH rats.

Benign arterial lesions were found in the majority of the FH rats: hyperplastic arteriolosclerosis was observed even in a few animals with hardly any glomerular or tubular changes. Similarly in human patients with essential, non-malignant, hypertension it was noted that the consistent arterial and arteriolar hyperplasia and/or hyalinization was accompanied only occasionally with glomerular changes (Jones 1974).

Hypertension-induced vascular obstruction, or hypertensive renal parenchymal disease, may stimulate renin release and cause additional renal hypertension. Luminal narrowing by intimal or medial hyperplasia may appear anywhere along the renal artery tree, causing partial ischaemia of a kidney segment. Animal 14, for example, having a very high PRA value, showed obliteration of a major branch of the renal artery (Fig. 6).

There is strong evidence that renin plays a major role in inducing malignant vascular lesions. Parenterally administered renin or angiotensin raises blood pressure, increases vascular permeability and evokes malignant arterial changes such as fibrinoid necrosis (Masson *et al* 1962; Giese 1964; Cuthbert *et al.* 1966; Robertson & Khairallah 1973).

In experimental hypertension, induced in rats by aortic ligation between the renal arteries, vascular permeability was increased only during the high-renin phase of the hypertension (Gabbiani & Elemer 1978). Chatelain *et al.* (1980), using the same experimental model, found that those rats developing malignant hypertension upon aortic ligation had similarly increased blood pressure values but markedly increased PRA values compared with the rats developing benign hypertension after aortic ligation. The rats having malignant hypertension developed widespread necrotizing arteritis with massive fibrinoid deposition, whereas those with benign hypertension, having similar blood pressure values but normal PRA values, did not show necrotizing lesions.

Helmchen and Kneissl (1981) developed a model of maximal stimulation of the renin angiotensin system (RAS) in rats remaining normotensive. Stimulation of the RAS was achieved by inducing hypovolaemia through subcutaneous oedema, evoked by administration of polyethylene glycol solution. Within 24 h fibrinoid necrosis of renal arteries and arterioles developed without hypertension, similarly suggesting that for the development of malignant vascular changes renin is of major importance.

As with hypertensive vascular lesions, high capillary blood pressures as well as increased renin levels may play a role in the development of glomerulosclerosis, the glomerulus being considered a microvascular system. Furthermore, glomerular (segmental) ischaemia, caused by preglomerular or intraglomerular vascular obstruction, contributes to the development of glomerulosclerosis and will simultaneously increase the PRA secretion. The close relationship between PRA values and glomerulosclerosis scores in 40-week-old animals could therefore be expected.

Several animals with malignant renal pathology had relatively low PRA values as compared to benign hypertensive animals. In these animals malignant changes cannot be ascribed to increased PRA levels in the systematic circulation. Helmchen and Kneissl (1981), however, report that the intrarenal renin content may increase enormously in case of acute stimulation of the RAS. In this situation, intrarenal renin causes malignant intrarenal arterial and arteriolar lesions, but it does not affect other organs; the plasma renin levels in the systemic circulation remain in the normal range.

If the RAS is important for the development of malignant vascular lesions, as sup-

ported by our present findings in older spontaneously hypertensive FH rats, drugs that antagonize the RAS should be effective in the treatment or prevention of malignant hypertensive disease.

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