Kidney International, Vol. 46 (1994), pp. 1528–1532

Spontaneous development of malignant phase hypertension in transgenic Ren-2 rats

CAROLINE E. WHITWORTH, STEWART FLEMING, ALLAN D. CUMMING, JAMES J. MORTON, NICOLA J.T. BURNS, BRENT C. WILLIAMS, and JOHN J. MULLINS

Centre for Genome Research, Department of Pathology, and Department of Renal Medicine, University of Edinburgh; MRC Blood Pressure Unit, Western Infirmary, Glasgow; and Department of Medicine, Western General Hospital, Edinburgh, Scotland, United Kingdom

Spontaneous development of malignant phase hypertension in transgenic Ren-2 rats. Spontaneous development of malignant phase hypertension in TGR(mREN2)27 heterozygotes occurs as a consequence of crossing TGR(mREN2)27 homozygotes with Edinburgh Sprague-Dawley rats. Similarities to human malignant phase hypertension are seen with an accelerated rise in blood pressure, fibrinoid necrosis of renal afferent arterioles, renal failure and evidence of renin-angiotensin system activation. It appears that introduction of an additional genetic factor or factors into a monogenic model of hypertension results in malignant phase hypertension.

Malignant hypertension (MH) is a rare but important complication of human essential hypertension [1, 2]. Clinical markers of transformation to the accelerated phase include a rising blood pressure, presumed pressure diuresis, renal failure and development of grade III or IV retinopathy. Characteristic pathological changes in the renal vasculature occur including myointimal proliferation or 'onion skinning' with endothelial swelling and fibrinoid necrosis [3, 4]. Activation of the renal renin-angiotensin system (RAS) is a presumed consequence of salt and water loss combined with afferent renal vascular pathology. This may serve to further increase blood pressure resulting in a vicious circle of progressive renal damage and rising blood pressure.

Most animal models of MH to date have been limited by the need for dietary, surgical or pharmacological intervention to precipitate onset and this has made the study of both primary or initiating factors and secondary events operating in malignant hypertension difficult to study [5–7].

The transgenic rat line TGR(mREN2)27 develops hypertension as a consequence of introduction and expression of the mouse Ren-2 renin gene into the Sprague-Dawley (SD) rat [8]. Blood pressure rises to a plateau at 10 weeks of age and development of progressive left ventricular hypertrophy, arterial medial thickening and glomerulosclerosis occurs with increasing age [9]. Fibrinoid necrosis was not described in transgenic heterozygotes. However, the underlying pathogenesis of the hypertension remains unclear.

The breeding of a colony of TGR(mREN2)27 heterozygotes in Edinburgh using SD rats (Centre for Genome Research, University of Edinburgh) was associated with the spontaneous onset of a phenotypic change with the occurence of a fatal syndrome at 50 to 90 days of age. Features included a one to three day history of polyuria, weight loss, dehydration, apathy, piloerection, seizures and hemiplegia. Some similarities to human MH prompted this study to characterize this as a potential animal model and to identify whether environmental or genetic factors may be responsible.

Methods

Initially three transgenic heterozygote crosses were bred. Homozygote TGR(mREN2)27 rats (TGR#27), derived from a Hannover SD colony (Central Institute for Laboratory Animal Breeding, Hannover, Germany) were crossed with (1) SD (Edinburgh) obtained from the colony in the Centre for Genome Research, (2) SD (Hannover) from the Central Institute for Laboratory Animal Breeding (Hannover, Germany) and (3) Lewis rats from Harlan-Olac (Bicester, Oxford).

All animals were housed in the same room with a 12:12 hour light-dark cycle, controlled temperature (18 to 20°C), humidity (45 to 65%), diet (0.32% sodium CRM diet, SDS, Witham, Essex, UK) and *ad libitum* tap water to drink. An alternative standard rat diet containing 0.2% sodium (CRM X) was prepared, and when used was given from weaning.

Direct blood pressure monitoring using telemetry (Data Sciences International, St. Paul, Minnesota, USA) allowed continuous recording of mean blood pressure (MBP) in conscious, unrestrained male rats starting from 46 to 50 days of age following recovery from surgical implantation under halothane anaesthesia [10, 11]. Indirect measurement of systolic blood pressures (SBP) used a tail cuff plethysmography method (IITC Life Sciences) under light halothane anaesthaesia. Student's *t*-test was used for statistical analysis with a P value of less than 0.05 taken to be significant.

Light microscopic examination of kidney, heart, brain and mesenteric artery was performed after fixation of tissues in 4% formal saline, wax embedding, sectioning (3 μm) and staining (hematoxylin and eosin, Martius Scarlett Blue, and periodic acid schiff stains). Renal function was assessed by plasma creatinine (Electro-nucleonics®). Plasma renin activity (PRA), angiotensin II (Ang II) and aldosterone were assayed by specific radioimmunoassay [12–14]. Immunohistochemistry of fixed kidney sections

Table 1. The incidence of the MH phenotype in male and female transgenic heterozygotes maintained under the same environmental conditions and on the same diet

Heterozygote cross	Name	Incidence of MH phenotype	
		Male	Female
TGR#27-Edin SD	TGR/Edin	86/117 (73.5%)	83/158 (52.5%)
TGR#27-Han SD	TGR/Han	7/39 (18%)	2/44 (4%)
TGR#27-Lewis	TGR/Lew	0/35 (0%)	0/31 (0%)

was performed using a polyclonal rabbit anti-mouse renin antibody, which cross reacts with rat renin (provided by Dr. D.J. Campbell, Melbourne, Australia).

Results

The occurrence of the MH phenotype in male and female transgenic heterozygotes is given in Table 1, which shows a significantly higher incidence in TGR/Edin heterozygotes than either TGR/Han or TGR/Lew heterozygotes. The median age at the time of death was 59 days (range 46 to 102 days).

Continuous recording of blood pressure in conscious TGR/Han and TGR/Lew heterozygotes by telemetry showed development of hypertension with a characteristic rise to a plateau by 70 days of age with a MBP of 170 mm Hg (Fig. 1A). In contrast, those TGR/Edin (7 out of 10) and TGR/Han (1 out of 12) that developed MH while on telemetry showed a continuing increase in blood pressure, which culminated in a terminal accelerated rise of 67 to 75 mm Hg (mean 72 mm Hg) over six hours (Fig. 1B). MBP in pre-malignant phase TGR/Edin was significantly higher than either TGR/Han or TGR/Lew at 52 to 57 days of age, but by 75
days of age the MBP attained by TGR/Han and TGR/Lew heterozygotes was not significantly different to pre-malignant phase TGR/Edin (data not shown).

All animals that exhibited signs of the MH phenotype were found to have pathological changes in the kidney. Figure 1C shows a kidney section from an 8-week-old hypertensive, but not malignant phase, transgenic rat kidney. In contrast, Figure 1D shows changes of MH. Fibrinoid necrosis was seen to principally affect afferent arterioles, interlobular arteries and occasional portions of glomerular tufts. Secondary ischemic changes were relatively rare, implying that such changes were acute. In addition proliferative myointimal changes with endothelial swelling and luminal thrombi were observed. More chronic hypertensive vascular and glomerular changes were not seen in the age range of the animals developing malignant phase hypertension. Small myocardial microinfarcts were seen in many TGR/Edin that developed MH, some in relation to fibrinoid changes within small cardiac arterioles. Cerebrovascular pathology was infrequent, with occasional infarction and hemorrhage. Minor thickening was seen in mesenteric artery wall, but there was no difference between malignant and non-malignant phenotypes.

The MH phenotype was associated with varying degrees of renal failure with a mean creatinine of 111.9 μmol/liter in malignant TGR/Edin compared to 55 healthy age-matched hypertensive heterozygotes (range 22.4 to 60.4 μmol/liter, 95% confidence limits). No significant difference in renal function was observed between the transgenic heterozygote crosses prior to the development of MH.

Significant elevation of PRA (29.1 $\pm$ 11.0 vs. 9.2 $\pm$ 8.3 ng Ang I/ml/hr), Ang II (829.5 $\pm$ 653.4 vs. 28.0 $\pm$ 25.2 pg/ml) and aldosterone (11.4 $\pm$ 7.7 vs. 0.71 $\pm$ 0.97 nmol/liter) were seen in MH rats compared with age matched, non-malignant phase, hypertensive transgenic heterozygotes (Results shown as mean $\pm$ SD, N = 6 to 24 per group; $P < 0.001$). Immunohistochemical labeling of kidney sections using a rabbit anti-mouse renin antibody demonstrated a marked increase in renin staining at the vascular poles in malignant hypertensives (not shown). Staining of other renal structures was not found.

A small reduction in dietary sodium (0.2% vs. 0.32%) within the 'normal' range was given from weaning to transgenic heterozygotes to mimic rat chow fed to original TGR/Han heterozygotes when first established and studied in Department of Pharmacology, University of Heidelberg, Germany [9]. There was a small reduction in MBP in telemetered TGR/Han and TGR/Lewis (Fig. 2 A, B) which was only significant at 75 days in TGR/Han and 75 to 81 days in TGR/Lew. No significant differences in the occurrence of MH occurred, with one case in the TGR/Han group on 0.32% sodium diet, but none in either TGR/Lew group.

SBP measured by tail cuff plethysmography under light halothane anaesthesia in non-transgenic Edinburgh SD and Hannover SD (N = 6 to 14 per group) on a 0.32% Na diet from 4 to 12 weeks of age showed a lower SBP in Edinburgh SD from six weeks of age and slightly lower body weight (Fig. 2 C, D)

Discussion

The spontaneous occurrence of the MH phenotype in the transgenic rat line TGR(mREN2)27 is associated with evidence of classical pathological changes of fibrinoid necrosis and myointimal proliferation, renal failure, evidence of renal RAS activation and an accelerated rise in BP. Furthermore, the high percentage of male TGR/Edin affected offers a potentially valuable animal model in which to study MH, in particular the initiating and subsequent events.

In this case transgenesis has created a monogenic model of hypertension in which additional interactions, either environmental or genetic, have resulted in MH. What factors determine susceptibility in humans are poorly understood. Cigarette smoking [15, 16], oral contraceptive use [17] and HLA type have all been implicated [18]. Environmental factors were questioned in view of the change in phenotype on breeding TGR#27 heterozygotes in Edinburgh as compared to Heidelberg. Analysis of rat chow revealed a slightly higher sodium content in 'Scottish' rat chow, but a comparison of the two diets did not significantly affect either blood pressure or survival. No other environmental differences such as temperature, humidity profiles or lighting times were found to explain the phenotypic change. Furthermore, the high incidence of MH in TGR/Edin compared with TGR/Han and TGR/Lew when maintained in identical conditions and on the same diet suggested that environmental factors were not crucial. It is likely that genetic diversity within the SD strain, which is outbred, explains the difference between Hannover and Edinburgh strains. The finding of a lower SBP in Edinburgh SD compared with Hannover SD rats argues against additional 'hypertensive' genes contributing to the MH phenotype.

Neither the rate of rise in blood pressure nor absolute BP appear to be important in determining the risk of onset of malignant phase hypertension, but higher BP at a younger age may be important in these rats. The continuing rise in BP during

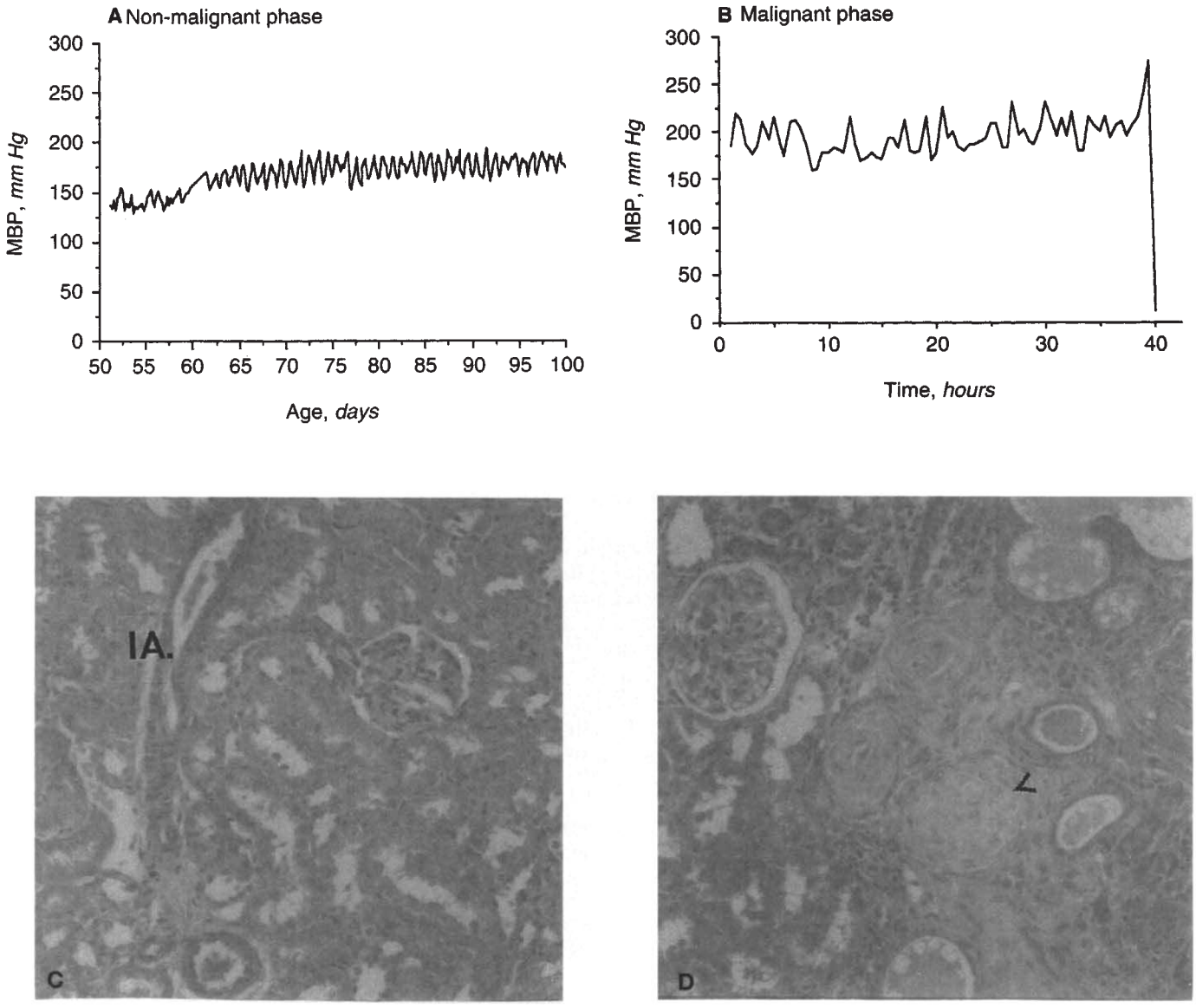

Fig. 1. *Two examples of continuous recording of MBP by telemetry show in* (**A**)*, 'normal' hypertension reaching a plateau phase of 170 mm Hg in a TGR/Han, and in* (**B**)*, the terminal 40 hours of a 79 day old TGR/Edin with MH demonstrating the accelerated phase with a rise of 75 mm Hg in six hours.* (**C**) A light micrograph showing a normal glomerulus, afferent arteriole and interlobular artery (IA) from a non-malignant phase transgenic heterozygote. (**D**) Myointimal proliferative changes (arrowed) and fibrinoid necrosis of interlobular arteries from a TGR/Edin with MH. (H&E stain ×100)

the accelerated phase may be due to loss of autoregulatory mechanisms and the development of a high renin hypertension. Initial characterization of the TGR(mREN2)27 rat line has suggested that the hypertension may be Ang II dependent [19], though there is evidence that altered adrenal steroid metabolism may be important [20, 21]. Certainly transgenic rats with established hypertension have evidence of a suppressed kidney RAS [8]. In the malignant phase, activation of the renal RAS was seen with increased immunohistochemical staining of the afferent arteriole at the vascular pole. By the method used, we have not differentiated mouse from rat renin, but would expect that as a consequence of the relatively low level of transgene expression in the kidney [8], that it is predominantly endogenous rat renin which is expressed in the malignant hypertensive transgenic rat kidney.

In conclusion, we believe that this may be a valuable animal model in which it may be possible to identify factors, either genetic or environmental, that contribute to the development of malignant phase hypertension. It suggests that the target organ damage resulting from hypertension in this case may in part be dependent on the interaction of additional genetic factors.

Reprint requests to Dr. C.E. Whitworth, Centre for Genome Research, University of Edinburgh, West Mains Road, Edinburgh EH9 3JQ, Scotland, United Kingdom.

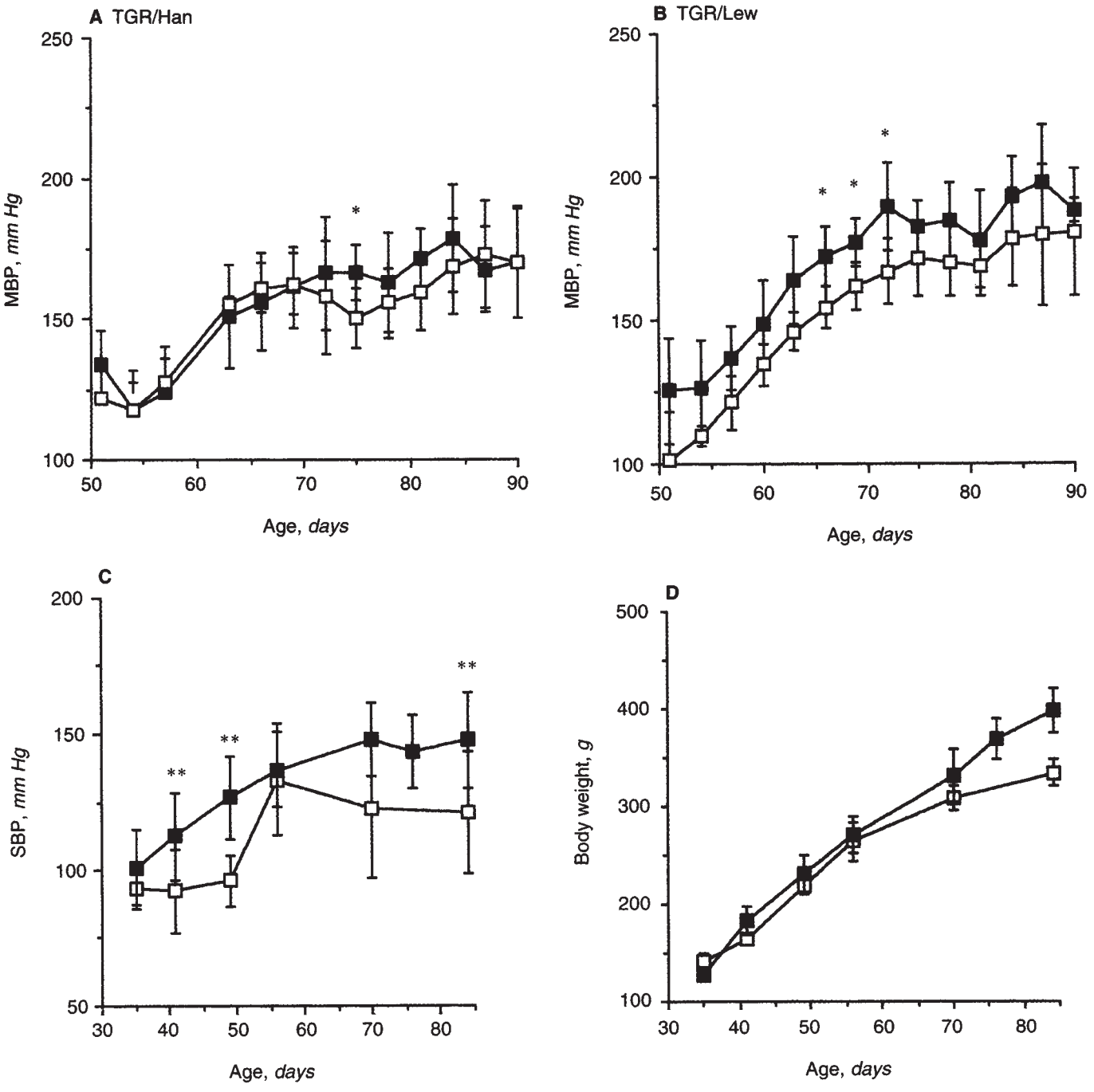

Fig. 2. *MBP (mm Hg) measured by telemetry in* (**A**) *TGR/Han* (N = 6, 5) *and* (**B**) *TGR/Lew heterozygotes* (N = 4, 5) *on 0.32%* (■) *and 0.2%* (□) *sodium diets, respectively.* Results are shown as mean ± SD for each group. MBP for each animal is taken as the mean over the 24 hour period (recordings at 10 min intervals). *Significant difference ($P < 0.05$) in MBP arising between 0.32% and 0.2% sodium diets. (**C**) SBP (mm Hg) of Edinburgh SD (□) and Hannover SD (■) rats from 4 to 12 weeks of age measured weekly by tail cuff plethysmography under light halothane anaesthesia; **SBP for Han SD > Edin SD ($P < 0.05$); (**D**) body weight.

References

1. KINCAID-SMITH P, MCMICHAEL J, MURPHY EA: The clinical course and pathology of hypertension with papilloedema (malignant hypertension). *Quart J Med* 27:117–153, 1957
2. HOUSTON MC: Pathophysiology, clinical aspects, and treatment of hypertensive crises. *Prog Cardiovasc Dis* 22:99–148, 1989
3. WILSON C, BYROM FB: Renal changes in malignant hypertension. *Lancet* 1:136–139, 1939
4. KINCAID-SMITH P: Renal pathology in hypertension and the effects of treatment. *Br J Clin Pharmacol* 13:107–115, 1982
5. BYROM FB: The nature of malignancy in hypertensive disease. Evidence from the retina of the rat. *Lancet* 1:516–520, 1963
6. LOHMEIER TE, TILLMAN LJ, CARROLL RG, BROWN AJ, GUYTON AC: Malignant hypertensive crisis induced by chronic intrarenal norepinephrine infusion. *Hypertension* 6:I.177–I.182, 1984
7. GAVRAS H, BRUNNER HR, LARAGH JH, VAUGHAN ED, KOSS M, COTE

LJ, GAVRAS I: Malignant hypertension resulting from deoxycorticosterone acetate and salt excess. *Circ Res* 36:301–309, 1975
8. MULLINS JJ, PETERS J, GANTEN D: Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene. *Nature* 344:541–544, 1990
9. BACHMANN S, PETERS J, ENGLER E, GANTEN D, MULLINS J: Transgenic rats carrying the mouse renin gene—Morphological characterization of a low-renin hypertension model. *Kidney Int* 41:24–36, 1992
10. LANGE J, BROCKWAY B, AZAR S: Telemetric monitoring of laboratory animals: An advanced technique that has come of age. *Lab Animal* 20:28–33, 1991
11. BIDANI AK, GRIFFIN KA, PICKEN M, LANSKY DM: Continuous telemetric blood pressure monitoring and glomerular injury in the rat remnant kidney model. *Am J Physiol* 265:F391–F398, 1993
12. MORTON JJ, WALLACE ECH: The importance of the renin-angiotensin system in the development and maintenance of hypertension in the two kidney one clip hypertensive rat. *Clin Sci* 64:359–370, 1983
13. MORTON JJ, WEBB DJ: Measurement of plasma Angiotensin II. *Clin Sci* 68:483–484, 1985
14. DAVIES E, ROSSITER S, EDWARDS CRW, WILLIAMS BC: Serotoninergic stimulation of aldosterone secretion in the rat in vivo: Role of the renin-angiotensin system. *J Endocrinol* 130:347–355, 1991
15. ISLES C, BROWN JJ, CUMMING AM, LEVER AF, MCAREAVEY D, ROBERTSON JS, HAWTHORNE VM, STEWART GM, ROBERTSON JWK, WAPSHAW J: Excess smoking in malignant-phase hypertension. *Brit Med J* 1:579–581, 1979
16. PATEL R, ANSARI A, GRIM C: Prognosis and predisposing factors for essential hypertension in predominantly black patients. *Am J Cardiol* 66:868–869, 1990
17. HODSMAN GP, ROBERTSON JIS, SEMPLE PF, MACKAY A: Malignant hypertension and oral contraceptives: Four cases, with two due to the 30 μg oestrogen pill. *Eur Heart J* 3:255–259, 1982
18. FORSBERG B, LOW B: Malignant hypertension and HLA antigens. *Tissue Antigens* 22:155–159, 1983
19. HILGERS KF, PETERS J, VEELKEN R, SOMMER M, RUPPRECHT G, GANTEN D, LUFT FC, MANN JFE: Increased vascular angiotensin formation in female rats harboring the mouse Ren-2 gene. *Hypertension* 19:687–691, 1992
20. SANDER M, BADER M, DJAVIDANI B, MASER-GLUTH C, VECSAI P, MULLINS J, CANTEN D, PETERS J: The role of the adrenal gland in hypertensive transgenic rat TGR(mRen2)27. *Endocrinology* 131:807–814, 1992
21. LO M, MEDEIROS I, MULLINS J, GANTEN D, BARRES C, CERUTTI C, VINCENT M, SASSARD J: High blood pressure maintenance in transgenic mREN-2 vs. Lyon genetically hypertensive rats. *Am J Physiol* 265:R180–R186, 1993