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Temporal Relationships of Treatment with Angiotensin Converting Enzyme Inhibitors on the Hypertension and Cardiac Hypertrophy in Spontaneously Hypertensive Rats (42088)

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Abstract. Two groups of spontaneously hypertensive rats (SHRs) were treated with captopril and enalapril, respectively. Treatment was started at weaning and at 8 months of age. Blood pressure was maintained close to normal in rats treated from weaning and significantly lowered but not to normal levels in rats started on treatment at 8 months. The long-term treated rats did not show significant cardiac enlargement whereas those treated at 8 months had an increase in cardiac size although not to the same level as untreated animals. Treatment with minoxidil and minoxidil plus propranolol similarly lowers blood pressure, but does not reduce myocardial hypertrophy. The data confirm that both the time of onset of treatment as well as the type of drug influence the myocardial complications of hypertension in SHRs. These studies further demonstrate the use of the SHR as a model for clinical pharmacologic studies with antihypertensive agents.

Considerable progress has been made in determining the need for antihypertensive therapy utilizing data derived most recently from large-scale clinical trials. However, a continuing question is whether a young hypertensive individual with mild disease and no target organ injury should be treated from the time of detection of hypertension or beginning in later years. The Hypertension Detection and Follow-Up Study (HDFP), for instance, does not demonstrate a clear-cut effect of treatment in patients under the age of 50 with mild hypertension (1), a finding also seen in the Australian trial (2). Although the risks of long-term treatment with modern-day antihypertensive agents may be small, nevertheless these risks are cumulative over time and certainly can magnify the direct and indirect costs of medical care. Accordingly, it would be worthwhile knowing to what extent cardiovascular consequences are affected by early versus late treatment. Conclusions have been derived mainly from extrapolation and speculation from short-term data, and to collect data in a prospective study in humans would be both pragmatically and ethically difficult to achieve.

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Although extending results from animals to clinical problems is controversial, the availability of the spontaneously hypertensive rat (SHR) strain, which shares many of the characteristics of essential hypertension in the human, has provided an attractive model to study in long-term pharmacologic experiments. Studies which have appeared include those by Freis and his colleagues (3) using reserpine, hydralazine, and thiazide, and by Takedo and Bunag (4) with propranolol in rather large doses (100 mg/kg/day) which are beyond those given to the human. An elegant series of studies by Sen (5) and Tarazi (6) have recently been summarized; these experiments, which include use of hydralazine, minoxidil, α-methyldopa and angiotensin converting enzyme inhibitors, have focused on the differential effects of these drugs on the mechanisms of left ventricular hypertrophy and its prevention. Similar studies concentrating on antiadrenergic drugs have been reported by Pegram and Frohlich (7). Weiss and Lundgren (8), Vavra *et al.* (9) and Richer *et al.* (10) have looked at several β-blocker drugs in the SHR, reporting varying results on blood pressure and cardiac size. There is a great variation in the drug doses used and duration of administration among all these studies.

For our own efforts in this area, we have employed potent present day drugs given

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chronically in doses *equivalent to those utilized in the human.* We have started therapy either at weaning of the rat at one month of age, or as late as 8 to 9 months of life, the latter representing essentially middle age in the lifetime of the SHR. Our major goal has been to determine if there is an *optimum time to begin therapy.* In preliminary experiments, rats were given either minoxidil alone, minoxidil plus propranolol, or the combination of minoxidil, propranolol and furosemide. The results of these pilot efforts will not be presented here since the outcomes were essentially similar to those reported by Sen (5) and Tarazi (6). These drugs lowered blood pressure, but even when begun at 1 month of age, and continued for 18 months, failed to significantly decrease left ventricular hypertrophy. Our report concerns low dose, long-term treatment with two currently available angiotensin converting enzyme inhibitors, namely captopril (E. R. Squibb and Sons) and enalapril (MK421, Merck & Co.). The experiments differed from those of Sen (5) using captopril in that we administered considerably smaller doses and continued the treatments for a longer period of time. Similarly, Richer *et al.* (16) and Sharma *et al.* (17) who have reported data with enalapril used much larger doses and treated for only 2 to 3 months.

General Methods and Materials. The SHRs used in these studies were from a colony maintained in our laboratory since 1975. These rats are originally from the Okamoto strain and were bred from several pairs obtained from the National Heart, Lung and Blood Institute colony. They have now been through six generations in our laboratory and become hypertensive usually by four months of age, eventually reaching levels as high as 200 mm Hg of systolic blood pressure with a life span of approximately 18 months. In the studies to be reported, systolic pressures were taken at 1-month intervals beginning at 1 month of age; they were recorded by the tail plethysmographic method as described in previous publications (11), through a sensitive transducer, with the rat under light ether anesthesia. The rats were housed in colony cages (five to a cage) and fed standard Purina rat chow. Drugs were administered in the daily drinking water supplied *ad lib* to the rats, at concentrations calculated to give the prescribed amounts of drug per kilogram per day, based on prior empirical determination of the amount of water consumed per rat. In each of the studies, the amount of water with the drugs in solution consumed daily by the treated rats was compared repeatedly during the experiment to the amount of water consumed by untreated control animals and no significant impairment of water intake produced by the drug solution was noted. Weights were recorded for each rat whenever blood pressure was determined and heart rates were determined from the plethysmographic tapes at each blood pressure measurement. At autopsy, blood was obtained by cardiac puncture, or following decapitation for rats in which plasma renin activities (PRA) were measured. Creatinine was determined by standard laboratory technique. PRA was measured by radioimmunoassay, utilizing the Haber method (12). Hearts were trimmed to the ventricles, weighed, and expressed as milligram percent body weight.

Statistical testing in the experiments with the angiotensin converting enzyme inhibitors was done with analysis of variance and covariance with repeated measures among the three groups in each experiment, with Student–Newman–Keuls t tests following analysis of variance for the endpoints data (13).

Specific Methods and Results. Since our preliminary experiments with minoxidil, propranolol, and furosemide had shown a fall in blood pressure, but no significant change in the heart size, even when the drugs were given for the lifetime of the rats, we decided to utilize drugs with less possibility of cardiac effects. We elected, therefore, to use angiotensin II converting enzyme inhibitors although elevation of renin activity has not been considered to play a major role in at least the initial development of the hypertension in the SHR (15). The dose of captopril was essentially equivalent to what has been given to humans (5 mg/kg/day) whereas Sen (5) had administered up to 30 mg/kg. The dose of MK421 was half as much as that of captopril based on information from its manufacturer that it is a longer acting and more potent compound. Richer *et al.* (16) and Sharma *et al.* (17) on the other hand have used doses of MK421 which are 10 to 40

times greater in SHRs and in Dahl rats, respectively.

In the captopril study (Experiment I), 61 male SHRs were randomly divided into 35 untreated controls and 26 treated animals. The treated group was started on therapy with captopril (5 mg/kg/day) immediately after weaning. At 8 months of age, 12 of the untreated controls were randomly selected and started on the same dose of captopril at that time. All rats were then followed for a total of 14 months, at which time survivors were sacrificed.

Of the 23 control rats (untreated throughout the study), 12 died but 6 of these deaths occurred within the first few months of the study and none appeared to be cardiovascular deaths. The other 6 died during the last few months of the study and all were hypertensive, some quite severely. However, of the 26 rats in the group receiving captopril throughout the study, there were 8 deaths, 4 of which were during the latter part of the experiment so that the differences in mortality could not be related to the variables under examination (e.g., hypertension and cardiovascular disease). Of the 12 rats switched to therapy at 8 months, only 1 died.

Figure 1 demonstrates the course of the blood pressure in the three groups of rats which survived to sacrifice. By the fifth month of the study, the treated group was significantly lower than the untreated animals and remained so throughout the 14 months of the study with final pressures which averaged 136 mm Hg. The group of 12 animals begun on treatment at 8 months had a significantly lower blood pressure than the untreated animals by 3 months later, but remained significantly higher than the long-term treated animals through the remaining months of the study.

Table I displays the endpoint data. The average heart weight was significantly less in the two treated groups than it was in the control untreated group but the difference between 328 and 352 mg/100 g body wt for the two treated groups did not reach a level of significance. (Average heart weight in normotensive rats in many studies in our laboratory is 250 to 300 mg/100 g body wt (14).) Body weights were identical throughout the study in all three groups. PRA levels, as expected from converting enzyme inhibition, were higher in groups that were treated than in the untreated controls, but did not reach a level of significance in this experiment. Creatinine levels were essentially identical in all three groups.

In the MK421 study (Experiment II), 64 male SHRs were randomly divided into 32 untreated controls and 32 treated experimen-

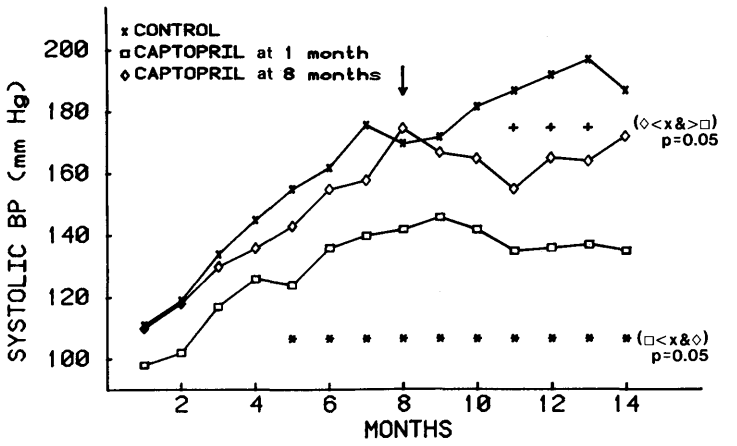

FIG. 1. Course of blood pressure in two groups of SHRs treated with captopril beginning at 1 month of age (□) and beginning at 8 months of age (◇), as contrasted with control, untreated rats (×). Rats treated with captopril throughout the 14 months had blood pressures sustained at essentially normal levels which were significantly different from controls. Blood pressures of rats beginning treatment at 8 months became significantly lower than controls, but remained significantly higher than the long-term treated rats.

TABLE I. EXPERIMENTS I AND II

	Number started	Deaths <8 (months)	Deaths 8–13 (months)	Final BP (n) (mm Hg)	Body weight (gm)	Heart size (mg 100 g body wt)	Renin activity (ng/ml)	Creatinine (mg/dl)
				Captopril (Expt. I)				
Controls (C)	23	6	6	186 (11)	398	388	23.5	0.64
Cap at 8 months (CE)	12	—	1	171 (11)	412	352**	43.4	0.66
Cap at 1 month (E)	26	4	4	136 (18)*	395	328**	36.6	0.64
				MK421 (Expt. II)				
Controls (C)	20	1	1	173 (18)	411	376	12.6	0.56
MK at 8 months (CE)	12	—	0	159 (12)**	414	347**	32.3	0.54
MK at 1 month (E)	32	4	1	136 (27)*	408	297*	50.3***	0.54

Note. n = Number of rats in group at termination; Cap = Captopril; MK = MK421 (Enalapril).
* Significantly less than C and CE.
** Significantly less than C.
*** Significantly greater than C.

tals and the latter started on 2.5 mg/kg/day of MK421 immediately upon weaning. Twelve of the controls were randomly selected at 8 months and started on therapy at that time, and again all three groups were followed for a total of 14 months before sacrifice.

Administration of MK421 yielded essentially the same results as with captopril. Mortality rates in all groups were lower. The untreated rats experienced only two deaths, both of which were early in the experiment. In the rats treated throughout the study, five deaths occurred, again from probable noncardiovascular causes, since four of them died in the first few months of the study; 27 survived to sacrifice. The 12 rats in whom treatment was started at eight months all survived to sacrifice.

The blood pressure results shown in Fig. 2 indicate that already at 4 months of age

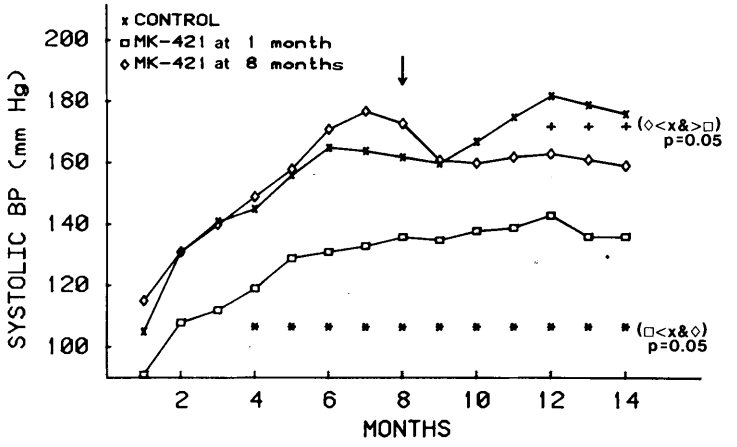

FIG. 2. Comparison of long-term treatment with MK421, beginning at 1 month of age and beginning at 8 months of age, as compared to controls. Symbols and interpretation as described for Fig. 1.

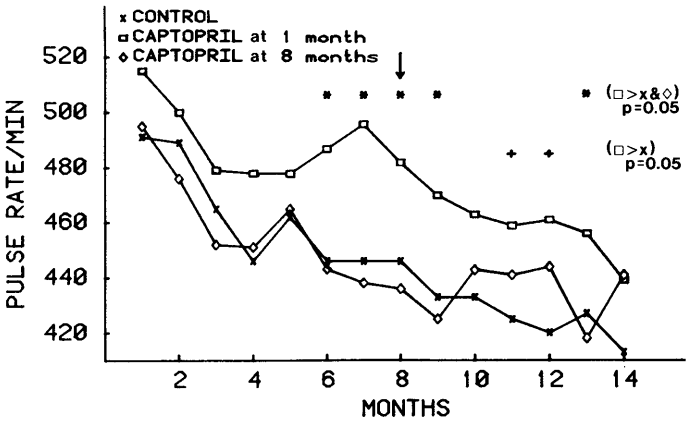

FIG. 3. Effect of captopril treatment on heart rate. Rats treated beginning at 1 month of age (□) showed a consistently higher heart rate than control rats (×). Rats starting treatment at eight months of age (◇) showed a slight rise as contrasted with controls, but remained lower than the long-term treated group.

the treated animals were significantly lower than the untreated animals and remained at virtually normal levels throughout the study, averaging 136 mm Hg at sacrifice at 14 months. The animals started on treatment at 8 months subsequently showed a gradual fall in blood pressure and during the last 3 months of the study were significantly lower than the untreated controls. Again, however, they remained higher than the animals treated throughout the study.

As shown in Table I, average heart weight in the rats treated with MK421 throughout the study was significantly lower than in either of the other two groups, namely 297 mg/100 g body wt, which approaches that of normotensive rats. The weight of 347 mg/100 g body wt in the group starting treatment at 8 months was significantly lower than that in the untreated controls (376 mg/100 g body wt) although higher than that of the rats treated throughout their lifetime. Again, body weights did not differ significantly among the three treatment groups. Terminal PRA in both of the treated groups was higher than the untreated controls but was statistically significant only for the difference between long term treated rats and the controls. Creatinine levels again were not significantly different.

Heart rate data are shown in Fig. 3 for captopril and Fig. 4 for MK421. Heart rate

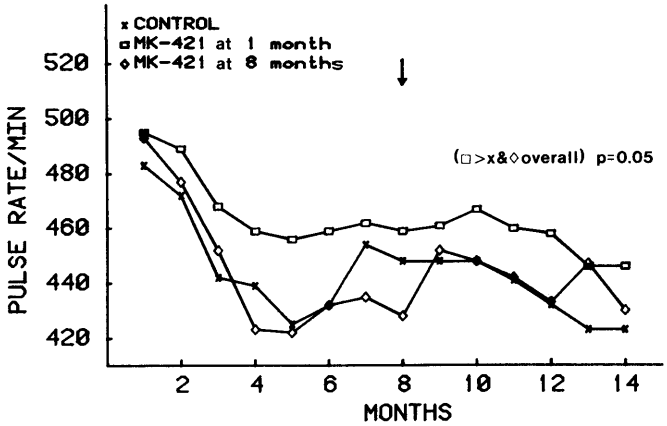

FIG. 4. Heart rate of rats treated with MK421 at 1 month of age were consistently faster than controls. Symbols and interpretations as Fig. 3.

was higher in rats treated with captopril throughout the study, although this difference largely disappeared in the short-term treated rats, after their treatment was initiated at 8 months. Similar results were seen for the MK421 study, with the long-term treated animals showing an overall higher rate than the other two groups.

Discussion. Our results with captopril and MK421 clearly indicate that it is possible to prevent or ameliorate the hypertension in the SHR when these medications are given in doses equivalent to those given to the human. Such therapy, begun early in the life of these rats, resulted in reduction of blood pressure over probably two-thirds of the lifetime of the animal and prevented myocardial hypertrophy. When effective medication was given later in life, blood pressure could be lowered, but not to the almost normal levels achieved with long-term therapy. Since our experiment was terminated somewhat earlier than the full-life expectancy of the animals, perhaps continued therapy beginning at the later time would further reduce pressure although our data do suggest that this reduction leveled off during the last few months of therapy. Under the circumstances of the later onset of therapy, cardiac hypertrophy was partially prevented but the size was not kept at the essentially normal level seen in the long-term treated animals.

Our results confirm the work of Sen (5), Richer *et al.* (16) and Sharma *et al.* (17) with ACE inhibitors and extend them by demonstrating effects with considerably smaller doses of the drugs. Moreover, we have shown maintained reduction of blood pressures throughout the 14-month life of the rats, with amelioration of cardiac hypertrophy; partial reversal of the hypertension, has been demonstrated even when the drugs were started as late as 8 months of age. In Sen's study (5) with captopril, treatment for prevention was for only 6 weeks and reversal was accomplished after only 8 weeks of hypertension. Richer *et al.* (16) studied only the prevention of hypertension; treatment with enalapril at 10 times the dose we used resulted in lower blood pressures after 3 months, which rose, but not to control levels, when treatment was discontinued. The studies with enalapril by Sharma *et al.* are also high dose and short duration and are concerned with Dahl rats and not SHRs.

Our pilot studies with minoxidil and propranolol, were in keeping with the findings of others (5, 6, 17) that lowering blood pressure of itself is not sufficient to prevent cardiac hypertrophy. As discussed by Tarazi (6), the type of medication used appears to be the important factor. Thus, when a potent vasodilator such as minoxidil is given to achieve lower blood pressure, the reflex cardiac stimulation and sodium retention which occur with this drug coincide with failure to reduce the ventricular hypertrophy. Moreover, when a β blocker is added to prevent adrenergic heart rate increase, cardiac function may deteriorate further, perhaps due to the added negative inotrophic effect of the β blocker along with its negative chronotropy. With Tarazi, we would emphasize the pertinence of these considerations to questions concerning choice of antihypertensive drugs in treatment of hypertensive subjects (6).

Captopril and MK421 were effective in reducing blood pressure in spite of the fact that the renin–angiotensin sequence is not considered to be the major cause of the hypertension in the SHR at least in its initial stages (15). These drugs, however, are modest diuretics because of their inhibiting effect on aldosterone production, which may have been at least partially the reason for their effect. Vasodilation is also suggested by slightly higher heart rates in the treated rats, possibly as a consequence of reflex tachycardia. Yet this increased heart rate did not prevent the decrease in cardiac weight, as it apparently may during treatment with both minoxidil and hydralazine, which are direct arterial smooth muscle dilators. Whether this inconsistency is a consequence of a difference in catecholamine accumulation in the heart also has been noted and discussed by Tarazi (6) and Sen (5).

It is of interest that no consistent impact on the renal function by hypertension or by treatment was noted in our studies, perhaps because renal complications are late events in the SHR (18) as they are in "benign" essential hypertension. However, we were using only a gross measure of renal function, namely, levels of serum creatinine, and it is possible that studies using more precise tech-

niques or studies extended for a longer period in the SHRs might reveal significant differences with drug treatment.

In fact, it is noteworthy that Feld *et al.* in their more recent studies in SHRs (19), extended to 100 weeks with blood pressure maintained at normal levels with reserpine, chlorothiazide, and hydralazine, noted that proteinuria nevertheless continued to increase, C_{cr} was not improved and glomerular injury was present. These investigators argue that a fundamental renal defect is present in the SHR, independent of blood pressure level; however, as with the heart size changes, the use of different pharmacologic agents for blood pressure control might lead to varying outcomes with respect to renal function. On the other hand, in the previously noted study by Bagby *et al.* (15), in which renin activity levels in SHRs were normal during the development of hypertension, elevation of PRA did develop after 35 weeks. Creatinine elevation was also seen in these older animals, which the authors interpret as evidence that the late renal damage causes the late PRA elevation.

We did not observe significant cerebrovascular disease developing in our rats but they were of the standard SHR variety and not the "stroke prone" strain which also has been developed by Yamori (20). Again, insights into cerebrovascular complications with treatment might be achieved in such a strain. Certainly, with the need to establish more firmly the optimum time to start therapy, to evaluate therapeutic outcomes in regard to end organ pathology, as well as blood pressure, and to determine if outcomes are variably affected by different types of drugs, the use of the SHR, and similar models which are being developed in primates (21), should be pursued to answer long-term clinical pharmacologic questions.

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1. Five year findings of the Hypertension Detection and Follow-up Program. I. Reduction in mortality of persons with high blood pressure, including mild hypertension. (HDFP Cooperative Group, DHEW) JAMA **242**:2572–2577, 1979.
2. The Australian Therapeutic Trial in Mild Hypertension, Report by the Management Committee. The Lancet, pp1261–1267, 1980.
3. Freis ED, Ragan D, Pillsbury H, Matthews M. Alteration of the course of hypertension in the spontaneously hypertensive rat. Circ Res **31**:1–7, 1972.
4. Takeda K, Bunag RD. Chronic propranolol treatment inhibits sympathetic nerve activity and keeps blood pressure from rising in spontaneously hypertensive rats. Hypertension **2**:228–235, 1980.
5. Sen S. Regression of cardiac hypertrophy: Experimental animal model. Amer J Med **75**(3A):87–93, 1983.
6. Tarazi RC. Regression of left ventricular hypertrophy by medical treatment: Present status and possible implications. Amer J Med **75**(3A):80–86, 1983.
7. Pegram BL, Frohlich ED. Cardiovascular adjustment to antiadrenergic agents. Amer J Med **75**(3A):94–99, 1983.
8. Weiss L, Lundgren Y. Chronic antihypertensive drug treatment in young spontaneously hypertensive rats: Effects on arterial blood pressure, cardiovascular reactivity and vascular design. Cardiovasc Res **12**: 744–751, 1978.
9. Vavra I, Tom H, Greselin E. Chronic propranolol treatment in young spontaneously hypertensive and normotensive rats. Canad J Physiol Pharmacol **51**: 727–732, 1973.
10. Richer C, Boissier JR, Giudicelli JF. Chronic atenolol treatment and hypertension development in spontaneously hypertensive rats. Eur J Pharmacol **47**: 393–400, 1978.
11. Shapiro AP. Susceptibility of rats with DCA hypertension to experimental pyelonephritis and aggravation of DCA hypertension by renal infection. J Lab Clin Med **55**:715–725, 1960.
12. Haber E, Koerner T, Page LB, Kliman B, Purnode A. Application of radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J Clin Endocrinol Metab **29**:1349–1355, 1969.
13. Winer BJ. Statistical Principles in Experimental Design. New York, McGraw-Hill, pp77–85, pp298–379, 1962.
14. Shapiro AP. Experimental pyelonephritis and hypertension. In: Metcoff J, Ed. 14th Annual Conference Proceedings of the National Kidney Disease Foundation. Angiotensin Systems and Experimental Renal Diseases. Boston, Little, Brown, pp161–179, 1963.
15. Bagby SP, McDonald WJ, Maas RD. Serial renin-angiotensin studies in spontaneously hypertensive and Wistar-Kyoto normotensive rats. Hypertension **1**:347–354, 1979.
16. Richer C, Dousau MP, Giudicelli JF. MK421 and

prevention of genetic hypertension development in young spontaneously hypertensive rats. Eur J Pharmacol **79**:23–29, 1982.

17. Sharma JN, Fernadez PG, Kim BK, Idikio H, Triggle CR. Cardiac regression and blood pressure control in the Dahl rat treated with either enalapril maleate (MK421, an angiotensin converting enzyme inhibitor) or hydrochlorothiazide. J Hypertens **1**: 251–256, 1983.
18. Feld LG, Van Liew JB, Glaske RG, Boylan JW. Selectivity of renal injury and proteinuria in the spontaneously hypertensive rat. Kid Int **12**:332–343, 1977.
19. Feld LG, Van Liew JB, Brentjens JR, Boylan JW. Renal lesions and proteinuria in the spontaneously hypertensive rat made normotensive by treatment. Kid Int **20**:606–614, 1981.
20. Yamori Y, Ohta K, Horie R, Khtaka M, Nara Y, Ooshima A. A new model for cerebral thrombosis and its pathogenesis. Jap Heart J **20(I)**:343–345, 1971.
21. Angelakos ET. Evaluation of basal blood pressure in African green monkeys. In: Kalter SS, Ed. The Use of Non-human Primates in Cardiovascular Diseases. Austin, Univ. of Texas Press, pp245–247, 1980.

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