PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 178, 629–634 (1985)

Glomerular Filtration and Fluid Balance in Genetically Hypertensive Mice (42053)

WARREN L. ROSENBERG,* GUNTHER SCHLAGER,† AND JOSEPH F. GENNARO, JR.‡

Department of Biology, Iona College, New Rochelle, New York 10801; †Division of Biological Sciences, The University of Kansas, Lawrence, Kansas 66045; and ‡Department of Biology, New York University, Washington Square, New York, New York 10003

Abstract. The Schlager genetically hypertensive mouse has been shown to be a valuable animal model with which to study human essential hypertension. Previous studies have characterized renal morphology, juxtaglomerular index, hematocrit, prostaglandin levels, brain catecholamines, social behavior, and patterns of inheritance. The present study continues the phenotypic characterization of this animal model. Using desiccation, isotope dilution, and clearance, the total body water, extracellular fluid volume, and glomerular filtration rate (GFR) in hypertensive and normotensive animals during normal postnatal development were measured. Additionally, using an electron microscopic tracer, the relative permeabilities of the glomerular filter in these animals were assessed. The data indicate a volume expansion in the young hypertensive animals along with a reduction in GFR. As the animals mature the volume expansion in the hypertensives subsides and is eventually reversed resulting in a lower than normal fluid volume level. The significance of the reduced GFR in the hypertensives is also diminished with age although not to the same degree as that of the fluid volume. The indication of a reduced glomerular permeability may account for the above in light of Guyton's cascade hypothesis. © 1985 Society for Experimental Biology and Medicine.

Previous work in our laboratories (1–3) and in others (4, 5) has shown that the Schlager strain of genetically hypertensive mouse is a well-suited animal model for the study of human essential hypertension. We have reported finding structural alterations in the glomeruli of these animals which would be consistent with an expected reduction in the glomerular filtration rate (GFR) as predicted by Guyton in his cascade hypothesis for the initiation of renal-based hypertensions (6, 7). According to this hypothesis, hypertension may be the result of a cascade of events beginning with a decrease in the excretion of water followed by an increased extracellular fluid volume, plasma volume, venous return, cardiac output, total peripheral resistance and finally, elevated blood pressure levels. It is possible that this scenario may stem from a chronically depressed GFR.

In the present study, determination of GFR was made for the hypertensive animals as well as for normotensive controls along with measurements of extracellular fluid volume using values from the dilution and clearance of ^{51}Cr-EDTA. In addition, an attempt was made to determine the relative permeabilities of the glomerular basement membrane, the primary barrier to glomerular filtration, between the two groups.

Materials and Methods. Male mice of the Schlager strain, selected for high blood pressure (HBP) and age-matched normotensive control mice (NCM) of the same strain were used in this study. All animals, of varying ages, were comfortably caged, maintained on a diet of Purina Lab Chow and tap water *ad libitum*, and exposed to normal daily light cycles and a temperature of 27°C.

Systolic arterial blood pressures were determined according to modifications of the technique of Williams *et al.* (8) in which the blood pressure is taken to be that pressure at which blood flow is detected in the tail distal to an occluding pressure cuff. Blood pressures in animals of the hypertensive line averaged 152 mm Hg while the normotensive controls averaged 114 mm Hg.

As measurement of GFR in the mouse using the standard inulin clearance technique presents many difficulties which many influence the results, the simpler yet comparably efficient method described by Hackbarth and Lunebrink (9) was used. This procedure involves injecting 3 μCi of ^{51}Cr-EDTA (New

0037-9727/85 $1.50
Copyright © 1985 by the Society for Experimental Biology and Medicine.
All rights reserved.

England Nuclear) in a vehicle volume of 0.01 ml/10 g body weight into the tail vein of the unanesthetized animal. At 10, 20, 30, 40, and 50 min after injection, 50-μl samples of venous blood are obtained by puncture of the retrobulbar vein plexus with heparinized microhematocrit tubes. Radioactivity of the samples, corrected for hematocrit, was measured in a Packard 5260 auto-gamma scintillation spectrometer at a counting time of 1 min per sample. The mean counts per sample were converted to the natural log and plotted against the time of sampling (Fig. 1). The GFR was calculated from the linear regression line as the product of the rate of clearance (slope) and the initial volume of distribution of the tracer. The latter factor also provides a measurement of the extracellular fluid volume (ECFV). Determination of total body water was performed by whole-body desiccation in tared weighing bottles at 100°C.

Cambar and Gendre (10, 11) have shown that qualitative estimates of changes in glomerular permeability can be determined by measuring the rate of passage of the electron-dense tracer molecule, ferritin, into the basement membrane. To estimate glomerular permeability in the HBP and NCM the animals were injected with a solution of native ferritin (2× crystallized, cadmium free) intravenously in the quantity of 0.02 ml/10 g body weight, slowly over a period of 1 min. The tracer was allowed to circulate for 15 min after which the kidneys were fixed by carefully removing the renal capsule and dripping fixative onto the exposed kidney surface for 15 min. At the same time, a small volume of fixative was also injected into the renal parenchyma according to the procedure described by Reeves *et al.* (12). Perfusion fixation was avoided as it would wash the tracer out of the capillary lumina. The fixative used was 3% glutaraldehyde in 0.1 *M* Pipes buffer, pH 7.4. After the initial fixation, thin cortical segments were removed, cut into sections no greater than 1 mm^3 and allowed to fix for a further period of 1 week at 4°C in several changes of fresh fixative. After subsequent postfixation in 1% OsO_4 (in the same buffer) and en bloc staining with 2% aqueous uranyl acetate, the tissue was dehydrated through a graded series of acetone, embedded in Epon 812, sectioned, and viewed with a Philips EM-300 transmission electron microscope. Micrographs were taken of randomly selected peripheral capillary loops and printed at a final magnification of 157,000×. Quantitation was performed with an analog to digital graphic data analyzing system (Ladd Research Industries). The ferritin density (particle number/unit area)

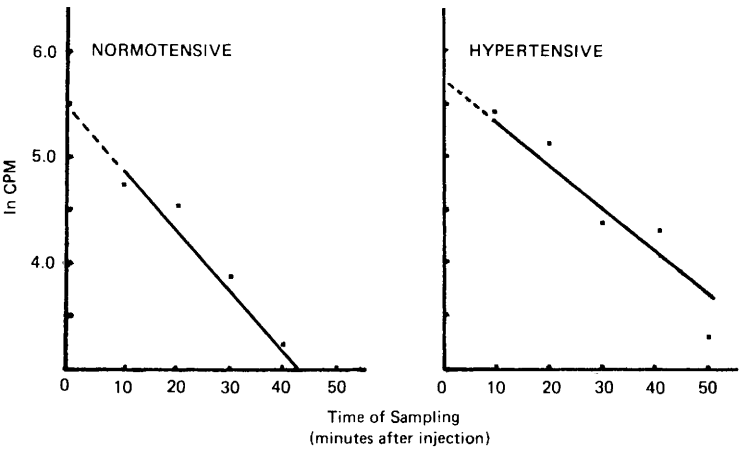

FIG. 1. Representative plots showing the rate of clearance of ^{51}Cr-EDTA from the plasma of mature normotensive and hypertensive male mice. From the clearance rate values glomerular filtration rate was calculated. The GFRs are expressed as ml/min/m^2 of body surface area. All data are $X \pm$ SEM. Normotensive: juvenile, 29.5 ± 3.7 (N = 4); mature, 42.5 ± 3.4 (N = 5). Hypertensive: juvenile, 20.7 ± 3.1 (N = 4); mature, 33.6 ± 3.6 (N = 5). P = <0.025 and <0.1 for juvenile and mature, respectively.

within the capillary lumen and glomerular basement membrane was determined and expressed as a ratio of basement membrane density/luminal density (10, 11).

Results. When the activity of ^{51}Cr-EDTA in the plasma is plotted against the time of sampling (Fig. 1), the rate of clearance of the tracer by the kidneys is reflected as the slope (K) of the linear regression line drawn through the sampling points. By extrapolation of this line back to time zero it is possible to determine the activity of the tracer after its initial dilution in the extracellular fluid compartment (A_0). The volume of the extracellular fluid compartment is determined as A_i/A_0, where A_i is the activity of the injected volume of tracer. The GFR is determined as the product of (A_i/A_0) (K) and standardized for body surface area differences (9).

The data indicate that there is a significant reduction in the GFR of the juvenile hypertensive animals (Fig. 1). The fluid volume in the juvenile animals of the hypertensive line shows an expansion to a degree significantly greater than that found in age-matched normotensive controls. As the animals age, the difference in the GFR and fluid volumes between hypertensive and normotensive animals decreases in significance with the fluid volume of the hypertensives ultimately becoming lower than in the normals (Figs. 2a, b).

Morphometric assessment of the relative permeability of the glomerular filter to the electron-dense protein tracer molecule, ferritin, shows a significant reduction in the permeability of the tracer through the glomerular filter of the superficial nephrons of the hypertensive animals (Fig. 3). There did not appear to be any significant difference between permeabilities in the juxtamedullary glomeruli.

Discussion. According to Guyton's cascade theory on the origin of hypertension (7) it is postulated that elevated blood pressure levels may result from an expansion of the ECFV, due primarily to a defect in the ability of the kidney to excrete a larger than normal water load. The possibility that this sequence of events may be brought about by factors which decrease the whole kidney GFR has been suggested (3). In such a cascade, a reduction in the GFR would lead to an increase in the extracellular fluid volume and hence, plasma volume, venous return, cardiac output, and arterial pressure would subse-

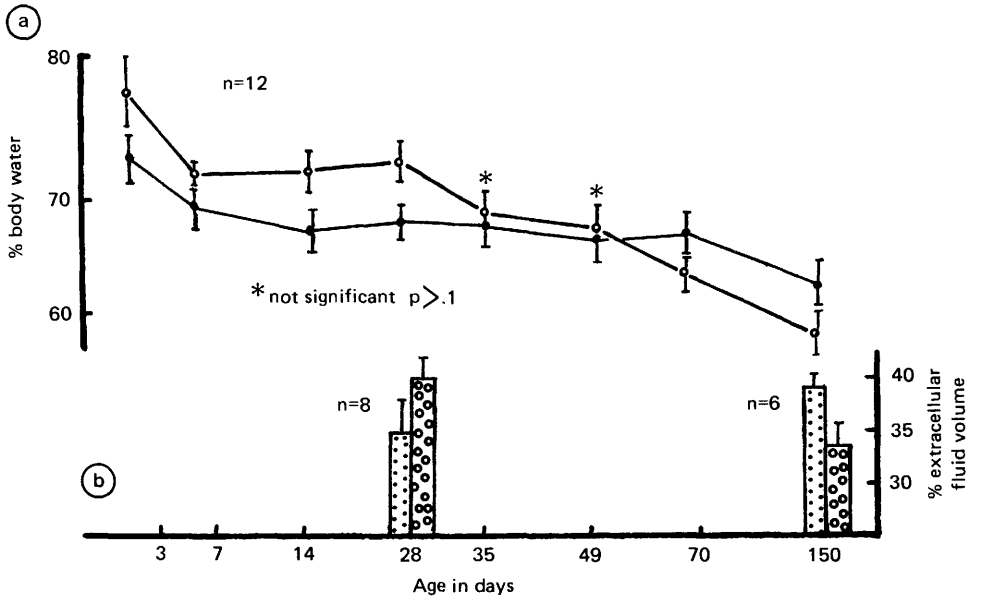

FIG. 2. Body fluid volume curves for hypertensive (○) and normotensive (●) male mice during normal development. Data show both total body water (2a) and extracellular fluid volume (2b) expressed as a percentage of total body weight.

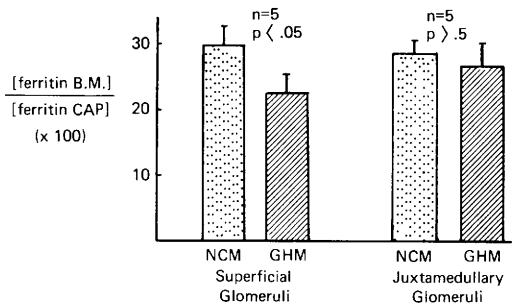

FIG. 3. Permeability of the glomerular basement membrane to ferritin. A decreased permeability in the hypertensive superficial glomeruli is reflected as a lowered ratio of the ferritin concentration in the basement membrane when compared to the concentration in the capillary lumen.

quently increase. The problem would be further compounded in that perfusion of the tissues with an excess of oxygenated blood due to the increased cardiac output would result in a constriction of the tissue arterioles through the process of autoregulation. This would increase the total peripheral resistance and further elevate blood pressure. Bianchi *et al.* (13) provided evidence of just such a response in Goldblatt hypertensive dogs. Reductions in GFR have previously been demonstrated in the spontaneously hypertensive rat (SHR) (14).

While one would expect such a response to be compensated for by glomerulotubular balance (15), it has been shown that this process is deficiently maladaptive by values of from 7 to 15% (16, 17). Guyton has shown that an increase in ECFV of only 2% would be sufficient to elevate blood pressure levels by values of from 35 to 65 mm Hg (18).

As determined by whole-body desiccation (Fig. 2a) it is apparent that there is an enhanced fluid retention in the hypertensive animals which develops early in life and which results in an increased extracellular fluid volume, evident by 28 days of age (Fig. 2b). As predicted by computer analysis (19) and demonstrated in experimental Goldblatt hypertension in dogs (20), the enhanced fluid retention in hypertension exists only during the initial rise of blood pressure, the volumes dropping later in the course of the disease (Figs. 2a, b). It is not uncommon for the ECFV to continue dropping with age so that in mature hypertensive individuals the fluid volume is often lower than that for age-matched normotensive individuals. The results of the present study confirm the existence of this phenomenon in the Schlager hypertensive mouse (Figs. 2a, b). This is not at all an unexpected finding and has been previously documented as occurring in human essential hypertensives (21–23). It is believed that the mechanism which initiates the rise in arterial blood pressure is not the same mechanism for maintaining the hypertension as the disease progresses. Recent evidence suggests that the maintenance of hypertension may be due in large part to the presence of a circulating sodium transport inhibitor released under the influence of the expanded ECFV (24, 25). The function of this sodium transport inhibitor would be to reduce tubular sodium reabsorption in the nephron preventing a further rise in sodium retention and fluid volume. However, at the same time this substance would inhibit sodium transport (extrusion) in the cells of all tissues, and would, in the arteries, lead to vasoconstriction and the maintenance of hypertension (26).

Clearance measurements with ^{51}Cr-EDTA indicate that the hypertensive mice have a significantly reduced GFR during the time of initial volume expansion with no significance apparent in the difference noted between the adults (Fig. 1). This reduced GFR and concomitant fluid retention in the juvenile animals might be the initiating factor in the onset of the hypertension. The absence of an expanded ECFV in the mature hypertensive animals in light of a somewhat lower but not significantly reduced GFR may be the result of the action of the postulated sodium transport inhibitor or of some other mechanism thought to maintain the chronic stage of hypertension.

Previous work in this laboratory has shown the presence of factors which may account for the reduction in GFR, namely a compression of the glomerular basement membrane (1) and a reduction in the available surface area for glomerular ultrafiltration (2). In the present study it also appears that the glomerular filter of the hypertensive animals is slightly less permeable than those of the

normotensive controls although only for the superficial cortical glomeruli (Fig. 3). The reduction in glomerular permeability as assessed with ferritin may be a contributing factor to the lower GFR and subsequent hypertension in these animals. Previous studies have shown the correlation between systemic hypertension and reduced glomerular permeability in pathologic and experimentally induced states (27–32).

As a result of the above findings, and in accordance with the cascade hypothesis, it seems likely that the hypertension as it exists in the Schlager mouse, is due to a structural or compositional change in the glomerular filter. These changes which develop early in life and subsequently diminish GFR apparently lead to an expansion of the ECFV ultimately resulting in chronic hypertension.

1. Rosenberg WL, Schlager G, Gennaro JF Jr. A morphometric analysis of the glomerular capillary wall of a new strain of genetically hypertensive mouse. Anat Rec **195**:511–523, 1979.
2. Rosenberg WL, Schlager G, Gennaro JF Jr. A structural basis for decreased glomerular function in genetically hypertensive mice. Anat Rec **202**:160A, 1982.
3. Rosenberg WL, Palmieri C, Schlager G, Gennaro JF Jr. Quantitative structural aspects of the renal glomeruli of hypertensive mice. Nephron **30**:161–165, 1982.
4. Schlager G. Selection for blood pressure levels in mice. Genetics **76**:537–549, 1974.
5. Sustarsic DL, McPartland RP, Rapp JP, Schlager G, Tan SY. Urinary kallikrein and urinary prostaglandin E-2 levels in genetically hypertensive mice. Proc Soc Exp Biol Med **163**:193–199, 1980.
6. Guyton AC, Coleman TG, Cowley AW, Manning RD, Ferguson JD. A systems approach to understanding long range arterial blood pressure control and hypertension. Circ Res **35**:159–176, 1974.
7. Guyton AC, Coleman TG, Cowley AW, Scheel KW, Manning RD, Norman RA. Arterial Pressure Regulation. In: Laragh JH, ed. Hypertension Manual. New York: Dunn-Connelly, Vol III, pp584–594, 1974.
8. Williams JR, Harrison TR, Grollman J. A simple method for the systolic arterial blood pressure of the unanesthetized rat. Clin Invest **18**:373–376, 1939.
9. Hackbarth VH, Lunebrink J. Die Bestimmung der glomerularen Filtrationsrate der maus mittels Totalclearance von ^{51}Cr-EDTA bie Fallendem plasmaspiegel in Abhangigkeit von Alter und Geschlect. Z Versuchtierkd **21**:38–46, 1979.
10. Cambar J, Gendre P. Influence de la theophylline sur le passage transglomerulare des macromolecules de ferritine chez le rat. CR Seances Soc Biol **171**: 596–601, 1977.
11. Cambar J, Gendre P. Etude du passage transglomerulaire des macromolecules proteiques chez le rat: Essai d'evaluation quantitative au moyen de la ferritine. CR Seances Soc Biol **171**:350–354, 1977.
12. Reeves WH, Kanwar YS, Farquhar MG. Assembly of the glomerular filtration surface: Differentiation of anionic sites in glomerular capillaries of newborn rat kidney. J Cell Biol **85**:735–753, 1980.
13. Bianchi G, Fox U, Piagetti D, Caravaggi AM, Baer PG, Baldoli E. Mechanisms involved in renal hypertension. Kidney Int **8**(Suppl):s165–s173, 1975.
14. Brink HM, Moons WM, Slegers JFG. Glomerular filtration in the isolated perfused kidney: II. Glomerular hemodynamics. Pfluegers Arch **397**:48–53, 1983.
15. Puschett JB, Goldstein S, Goodshall S, Staum BB, Goldberg M. Effects of filtration rate and plasma sodium concentration on proximal sodium transport. Amer J Physiol **221**:788–794, 1971.
16. Arrizurieta-Muchnik EE, Lassiter WE, Lipham EM, Gottschalk CW. Micropuncture study of glomerulotubular balance in the rat kidney. Nephron **6**:418–435, 1969.
17. Landwehr DM, Schneerman J, Klose RM, Giebisch G. Effect of reduction in filtration rate on renal tubular sodium and water reabsorption. J Physiol **215**:687–695, 1968.
18. Guyton AC, Coleman TG, Cowley AW, Scheel KW, Manning RD, Norman RA. Arterial pressure regulation. Amer J Med **52**:584–594, 1972.
19. Guyton AC, Coleman TG. Quantitative analysis of the pathophysiology of hypertension. Circ Res **24**(Suppl):1–19, 1969.
20. Bianchi G, Tenconi LT, Lucca R. Effect in the conscious dog of constriction of the renal artery of a sole remaining kidney on haemodynamics, sodium balance, body fluid volumes, plasma renin concentration and pressor responsiveness to angiotensin. Clin Sci **38**:741–766, 1976.
21. Brown JJ, Dusterdieck R, Fraser R, Lever AF, Robertson JIS, Tree M, Weir RI. Hypertension and chronic renal failure. Brit Med Bull **27**:128–135, 1971.
22. Guyton AC. Personal views on mechanisms of hypertension. In: Genest J, Kiow E, Kuchel O, eds. Hypertension: Physiopathology and Treatment. New York, McGraw-Hill, pp566–575, 1977.
23. Ledingham JM. Mechanism in renal hypertension. Proc Soc Med **64**:17–26, 1971.
24. Haddy FJ, Overbeck HW. The role of humoral agents in volume expanded hypertension. Life Sci **19**:935–948, 1976.

25. Louis F, Favre H. Natriuretic factor in rats acutely expanded by ringers versus albumin solution. Kidney Int **18**:20–28, 1980.
26. Blaustein MP. Sodium ions, calcium ions, blood pressure regulation and hypertension: A reassessment and a hypothesis. Amer J Physiol **232**:c165–c173, 1977.
27. Selkurt EE, Abel FL, Edwards JL, Yum MN. Renal function in dogs with hypertension induced by immunologic nephritis. Proc Soc Exp Biol Med **144**: 295–303, 1973.
28. Azar S, Tobian L, Johnson MA. Glomerular, efferent arteriolar, peritubular capillary and tubular pressures in hypertension. Amer J Physiol **227**:1045–1050, 1974.
29. Azar S, Johnson MA, Hertel B, Tobian L. Single nephron pressures, flows and resistances in hypertensive kidneys with nephrosclerosis. Kidney Int **11**: 28–40, 1977.
30. Schweitzer G, Gertz KH. Changes of hemodynamics and glomerular ultrafiltration in renal hypertension in rats. Kidney Int **15**:134–143, 1979.
31. Savin VJ, Lindsley HB, Nagle RB, Cachia R. Ultrafiltration and glomerular capillary resistance in a model of immune complex glomerulonephritis. Kidney Int **21**:28–35, 1982.
32. Steiner RW, Tucker BJ, Gushwa LC, Gifford J, Wilson CB, Blantz RC. Glomerular hemodynamics in moderate Goldblatt hypertension in the rat. Hypertension **4**:51–57, 1982.

Received September 19, 1984. P.S.E.B.M. 1985, Vol. 178.
Accepted January 4, 1985.