concluded that erythropoietin is probably a polypeptide. In the presence of the non-precipitable proteins (by heat) of plasma, it is fairly stable to mild oxidation and reduction.

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Factors Affecting Development of Hypertensive Vascular Disease After Renal Injury in Rats.* (23556)

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Hypertensive vascular disease may result from various types of renal injury in the experimental animal and often is associated with primary renal disease in the human, but the responsible mechanisms remain unclear. Furthermore, following apparently identical renal injury, incidence and severity of hypertension show considerable variation; *i.e.*, in rats after figure-of-eight tie of one kidney and contralateral nephrectomy, hypertension ranging from mild and chronic form to severe and rapidly fatal disease may develop(1,2). Whether this variability is related directly to extent of renal injury is not readily apparent, since precise studies of its relationship to degree of impairment of excretory renal function, to hypertrophy of remaining renal tissue, or to other factors, have not been reported following this particular injury, and studied in only a limited fashion with other technics for renal injury in rats(3-5). The present study was undertaken to investigate this relationship of renal injury to severity in rats with a figure-of-eight tie of one kidney and contralateral nephrectomy. In addition, the effect of a behavioral disturbance devised to produce a chronic stress for the rat was evaluated.

Materials and methods. Fifty-four male Holtzman rats weighing 150 to 250 g were subjected to "figure-of-eight" tie of the right kidney and, a week later, to a left nephrectomy, according to the technic described by Grollman(1). The animals were lodged in groups of 8 to 10/cage and allowed free access to food and water. Under light ether anesthesia, systolic blood pressure was determined biweekly by tail plethysmographic method(6,7). Renal function was studied prior to sacrifice by testing phenosulfonphthalein (PSP) excretion and urine concentrating ability. PSP excretion was measured by injection of 10 ml of sterile 0.2% saline, intraperitoneally, followed one hour later by a second 10 ml with 1.5 mg of PSP dye; urine was collected over a 3-hour period and percentage of dye excreted determined spectrophotometrically. To determine urine concentration, rats were kept in metabolic cages without food and water for 48-hour period and osmolal concentration of samples col-

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TABLE I. Average Value of Blood Pressure, Renal Function, and Organ Size in 17 Rats with Renal Injury.

	Rt. Fig. 8; Lt. Np.		Intact control		
	Mean	S.D.	Mean	S.D.	p
Systolic B.P. (mm Hg) (avg after onset of hypertension)	146	13.8	110 *	10	.001
Systolic B.P. (mm Hg) (avg of maximum)	160	16.0			
PSP excretion (%)	49.9	13.5	62.9	10.1	.01
48 hr concentration (milliosmoles/l)	1773	611	2413	284	.001
BUN (mg/100 ml)	51.8	14.5	28.7	3.0	.001
Heart wt (mg/100 g)	324	46.2	252	17.8	.001
Kidney " "	619	129.2	648 †	54.3	
Adrenal " "	15.2	2.8	11.1	1.5	.001

* This blood pressure is the avg obtained from several hundred normal animals in this laboratory. Consistent or even sporadic elevations to 140 mm Hg or over, have been rarely observed in our experience nor by other investigators employing this method(8,9).

† Wt of 2 normal kidneys.

lected between 24 and 48 hours measured in a Fiske osmometer. Rats were sacrificed by administration of nembutal and rapid exsanguination by cardiac puncture. Blood urea nitrogen (BUN) was determined on heart blood. The heart, kidney and adrenals were cleaned of extraneous tissue and weighed on a Sartorius balance. The kidney was fixed in 10% formalin, and sections prepared with hematoxylin-eosin, and Mallory stains. The 54 animals were divided into a control group of 24 and a stressed group of 30. Beginning 7 to 10 days after nephrectomy, animals in the stressed group were isolated in cubicles measuring 7 cubic inches for 6 hours 3 times a week. The floor of each cubicle consisted of a brass grid and buzzer was attached to top of each unit of 12 cubicles. Grid and buzzer were activated by a cam-driven automatic timing mechanism. During each 6 hour period in the cubicles, the following pattern was repeated 3 times: For one hour, the buzzer (conditioned stimulus) was sounded at 30 second intervals for 5 seconds, followed, with a 0.5 second overlap, by mild non-convulsive shock (unconditioned stimulus) delivered through the grid. For the next hour, CS was presented alone at 30 second intervals. The weekly procedure was administered for 12 weeks, followed by 6 weeks of rest and then repeated: in animals which survived to end of study, stress was discontinued 3 weeks prior to sacrifice so that renal function tests could be performed. After several trials in the apparatus the animals responded to CS with a "startle" reaction and later in the experiment often appeared to "freeze" in one position. These responses were irregularly extinguished during the period when CS alone was sounded. The control group was kept in colony cages in the same experimental room, and thus experienced the noise of the buzzer, but it produced no apparent response in them.

Results. Thirty-seven animals, including 12 (33%) which failed to become hypertensive, died before completion of the study; their average post-operative life was 20 weeks. The psychological stimulation did not influence mortality rates; of the 37, 21 (70%) were stressed, and 16 (67%) were not. Seventeen animals, 9 under stress and 8 controls, survived with an average post-operative life of 36 weeks. All 17 surviving animals became hypertensive and showed evidence of renal impairment; the average values are summarized in Table I and compared with those from a similar number of normal animals of same age and source as the experimental group. Impaired PSP excretion and decreased osmolar concentration correlated with elevated BUN (Fig. 1). The ligated kidney hypertrophied but failed to compensate for impaired renal excretory function. Actually animals with the most hypertrophy demonstrated greatest impairment of concentrating ability and highest BUN (Fig. 2). Levels of blood pressure did not demonstrate a linear correlation with any of the renal function measurements or with renal mass, with the exception of a sug-

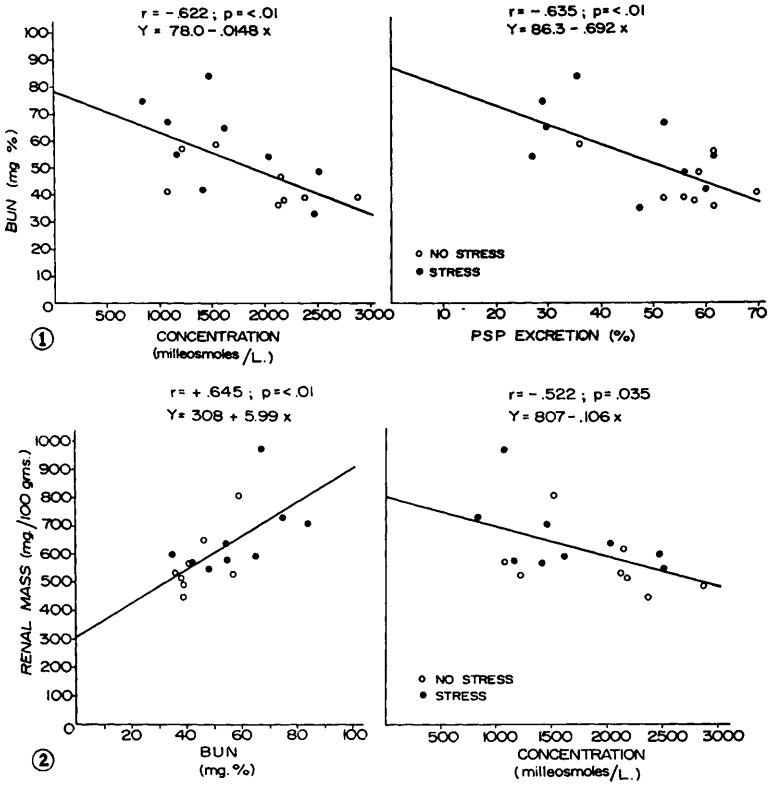

FIG. 1. Graphs indicate the negative correlations between BUN and concentrating ability, and BUN and PSP excretion. Each point represents one rat; stressed and non-stressed animals have been combined to form one group for these comparisons, but are charted with different symbols.

FIG. 2. Graphs indicate positive correlation between wt of the kidney (renal mass) and BUN, and negative correlation between renal mass and concentration. Each point represents one rat.

gestively positive relationship between maximum blood pressure and renal weight ($r = +.482$; $p = .05$). Although levels of blood pressure in both stressed and nonstressed animals did not differ, the severity of hypertensive disease as evidenced by heart size, renal excretory function and adrenal size, seemed more pronounced in the former (Table II). The onset of hypertension, as calculated from the number of weeks after surgery when a systolic blood pressure of 140 mm Hg or over was first noted, averaged 14.1 wks. in the stressed and 25.5 wks. in the nonstressed group. A 4-fold test of contingency(10), revealed a difference significant at the 5% level between number of animals in each group who were hypertensive at the 20th week. Duration of hypertension did not account for differences between stressed and

TABLE II. Comparison of Onset, Duration, and Severity of Hypertension in Operated Rats with and without "Psychological Stress."

	Stressed		Nonstressed	
No. of animals→	9		8	
	Mean	S.D.	Mean	S.D.
Avg age at sacrifice (wk)	47.7	13.8	47.3	13.1
Avg post-operative life (wk)	36.8	11.8	35.8	9.9
Avg systolic B. P. (mm Hg)	142	16.9	150	8.6
Avg max systolic B.P. (mm Hg)	159	19.0	161	12.9
Heart wt (mg/100 g)	349	38.7	296	37.5 *
Kidney wt "	663	133	570	113
Adrenal wt "	17.0	2.58	13.3	2.14*
BUN (mg/100 ml)	58.3	15.8	44.4	8.9 †
48 hr conc. (milli-osmoles/l)	1614	658	1941	601
PSP excretion (%)	44.1	14.1	56.5	9.70†

* Significant differences at 1% level.
† Probably significant differences at 5% level.

nonstressed groups, since it did not demonstrate a correlation with measurements of organ weight, renal function, or blood pressure ($p > .05$ in all instances).

Pathological changes. All components of the kidney nephron were affected, but the damage was most consistent in the tubules. Flattening of the epithelium in the proximal tubules, dilatation of distal and proximal elements, and accumulation of eosinophilic material in their lumens were noted. Scattered infiltrates of mononuclear cells were present throughout the kidney, but never to the degree nor with the scarring seen in experimental pyelonephritis in the rat(11). The glomeruli were well preserved and demonstrated only periglomerular fibrosis and hypertrophy until overall damage was moderate to severe, and then varying degrees of fibrosis ranging to complete replacement were noted. In the mildly affected kidneys, arteriolar damage was absent, but medial thickening and hyalinization were present in the moderate to severe group while in the worst kidneys intimal thickening, and occasionally typical "onion peel" lesions of nephrosclerosis, were apparent. Classification of the severity of damage to each kidney on the basis of 1+ to 3+ revealed the following:

	1+	2+	3+
Stress group*	0	4	4
Nonstress group	5	2	1

* Tissue was available for study in only 8 of the 9 animals in the stress group.

Discussion. Presence of excretory renal impairment and yet its failure to correlate directly with severity of hypertension is not a new or unexpected finding although the quantitative aspects of the relationship have not been previously demonstrated in rats with a figure-of-eight tie of one kidney and contralateral nephrectomy. The hypertrophy of the constricted kidney was striking, and it is of interest that it occurred despite injury to the kidney and that it failed to compensate for functional impairment. Nor did renal hypertrophy prevent the development of hypertension, indicating that if an increatory function of the kidney regulates blood pressure(12), it is not a function of renal mass. Since excretory renal impairment alone does not correlate directly with the severity of the hypertension, other factors must interact to determine the blood pressure regulatory function of the kidney and to influence the course of the disease. The seemingly adverse effects of the noxious psychological stimuli suggest one such factor. Ample clinical evidence indicates that psychological turmoil can play a role in the precipitation of acute changes in blood pressure and the progression of hypertensive vascular disease in man(13,14). The present experiment offers evidence in the experimental animal of these inferences.

Summary. Correlations between renal excretory function (PSP excretion, osmolal concentration of urine, and BUN), renal mass and hypertension were studied in 17 rats which had been subjected to a figure-of-eight tie of one kidney and contralateral nephrectomy. In addition, 9 of the animals were exposed to the stress of a psychological disturbance. All 17 animals became hypertensive and all had some impairment of excretory renal function. However, the degree of impairment of renal function did not correlate with the level of blood pressure. Hypertrophy of remaining renal tissue was marked but did not prevent functional impairment or blood pressure elevation. The noxious psychological stimulus appeared to worsen the manifestations of the hypertensive process.

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Effect of Physical Activity on Cholesterol Atherosclerosis in Rabbits.* (23557)

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Cholesterol atherosclerosis has been produced in animals by many investigators. The results of these experiments and of studies in the pathogenesis of the lesions have been extensively reviewed(1-4). In man the effect of diet on serum lipids has been extensively investigated. However, opinion varies on the importance of all lipids(5) and of the type of ingested lipid(17-21) causing variations of serum lipids in man. The serum lipid levels and the serum lipoprotein types have some relation to the occurrence of sequelae of atherosclerosis, but the predictive value and role of these factors in the pathogenesis of human atherosclerosis are not defined(7). In human beings, as well as in experimental animals, very little attention has been paid to the question of the influence of physical activity of the atherosclerotic process. The effect of physical activity on incidence of coronary artery disease in sedentary and more active workers was studied, and although the groups differed in respects other than those of physical activity, it was judged that the active workers had less severe heart disease than the more sedentary workers(6). Some indication was obtained that, following ingestion of a high fat meal, exercise lessened the degree of hypercholesterolemia(9). In studies of different national groups, a tendency to a lowered serum cholesterol was found in Bantus doing heavy manual labor, but in white populations, total fat content had more effect on cholesterol level in the blood than did exercise(8). In experimental animals there is, to our knowledge, only one study on record concerning the effect of physical activity on rabbits being fed cholesterol(10), in which the sedentary and exercised groups showed equal atherosclerosis. In our experiments an apparent decrease in the amount of atherosclerosis of the aorta of cholesterol-fed rabbits was shown to occur in the group of rabbits exercised in an electrified treadmill.

Materials and methods. The animals used in this experiment were New Zealand white rabbits purchased from a local supplier.‡ They consisted of 5 groups of 5 to 7 litter mates obtained from bucks and does of known pedigree. The animals were individually housed in air-conditioned animal quarters. Each litter of 5 to 6 animals was divided into 2 groups without reference to sex. One was fed cholesterol and allowed to remain sedentary. The other group was fed cholesterol but exercised. The sedentary and experimental groups are indicated in Table I and sex distribution is evident in Figs. 1-6. The inclusion of a group of animals fed an ordinary diet was not considered necessary since this strain of rabbits has been used for years in this laboratory for various purposes,

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