SODIUM EXCRETION IN CONSCIOUS UNRESTRAINED SPONTANEOUSLY HYPERTENSIVE (SHR) RATS AFTER ACUTE GANGLIONIC BLOCKADE

By

Edward E. Conrad, Jr.

A THESIS

Presented to the Department of Physiology
and to the Graduate Division of the University of Oregon
Health Sciences Center
in partial fulfillment of
the requirement for the degree of

Doctor of Philosophy
January 1979

APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

ACKNOWLEDGEMENTS

For that which is of value in this thesis, I owe much to the students and faculty of the Physiology Department, School of Medicine, University of Oregon Health Sciences Center. In particular, Drs. John M. Brookhart and Alfred J. Rampone have given me wise and kind support for all five years of my graduate education. I shall always be grateful to Dr. J. Job Faber for his encouragement throughout these years. He has always been available to discuss my problems as well as my progress. Dr. G.V.F. Seaman from the Department of Biochemistry introduced me to the methods and wonders of the life sciences. With him I learned the joy of discovery.

I am fortunate to have been guided in this work by two superb advisors. Dr. Walter J. McDonald from the Department of Medicine has always offered crucial suggestions about the methods and direction of my research. As both a clinician and a basic scientist he has been a model of the best in medicine and physiology. Finally, Dr. Robert E. Swanson from the Department of Physiology taught me the worth of precision in scientific work. For his loving friendship an unfailing support I am forever in his debt.

This work has been supported by funds from the Moses E. Steinberg Scholarship Fund, the Medical Research Foundation of Oregon (9925), the Oregon Heart Association and the National Institutes of Health (GM 00538).

DEDICATION

To Gina and Theodore
who continue to love and
support me in spite of
this thesis

ABSTRACT

There is no generally accepted theory for the pathogenesis of hypertension in the spontaneously hypertensive (SHR) rat. Various workers have, however, proposed that elevated activity of the sympathetic nervous system may be responsible for the genesis or maintenance of hypertension in the SHR rat. This study examined the role of sympathetic nerve activity in the maintenance of hypertension by studying the effect of acute ganglionic blockade on the arterial pressure-sodium excretion rate curve (the renal function curve) in conscious unrestrained rats.

I used female SHR rats (n=24) and normotensive Wistar-Kyoto (WKY) control rats (n=16) in three age groups: 8 to 12, 14 to 19 and 20 to 32 weeks old. The SHR rats were hypertensive relative to the WKY rats for all age groups and the blood pressure of SHR rats rose with age. Catheters were placed in the abdominal aorta, the external jugular vein and the urinary bladder under pentobarbital anesthesia. Experiments were performed at least 48 hours later in conscious rats. Acute ganglionic blockade and hypotension was produced with i.v. hexamethonium. The blood pressure was varied in some experiments by the infusion of fresh whole blood or by hemorrhage. After arterial pressure had stabilized, sodium excretion rate ($U_{Na}V$ - microEq$\cdot$min$^{-1}\cdot$100g BW^{-1}) was measured with 10 minute urine collections obtained by flushing the bladder. Mean arterial pressure (MAP - mmHg) was recorded simultaneously from the aortic catheter. Catheters remained functional for up to 6 weeks and it was possible to record as many as 15 pressure - $U_{Na}V$ points from a single rat.

Measurements of chronic sodium excretion rate and mean arterial pressure in this work agree with other reports that there is a rightward

shift of the chronic renal function curve in hypertensive SHR rats. In addition, the renal function curve after ganglionic blockade for each age group of SHR rats was to the right of the curve for age-matched WKY rats ($P< 0.01$). Within the same strain of rats, there was no difference in the positions of the renal function curves for each of the three age groups. Linear regression analysis was applied to the observations from all three age groups. The lines representing the renal function curves after ganglionic blockade for SHR rats (n=96 observations) or for WKY rats (n=86) were:

SHR: $U_{Na}V = -5.62 + 0.0653 \cdot MAP$ ($r=0.365$, $P< 0.001$)

WKY: $U_{Na}V = -6.05 + 0.1014 \cdot MAP$ ($r=0.359$, $P< 0.001$)

The two lines are significantly different (P 0.005) by an analysis of variance.

The persistence of the rightward shift of the SHR renal function curve after ganglionic blockade indicates that the sympathetic nervous system is not required for the maintenance of the rightward shift of the chronic renal function curve in hypertensive SHR rats. Since I argue that the rightward shift of the renal function curve is necessary for the maintenance of hypertension, these data suggest that the sympathetic nervous system is not solely responsible for chronic hypertension in the SHR rat.

These observations are consistent with the alternative hypothesis that structural changes in the renal vasculature are responsible for the rightward shift of the SHR renal function curve and may, therefore, be crucial for the maintenance of hypertension in the mature SHR rat.

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INTRODUCTION

PATHOPHYSIOLOGY OF HUMAN HYPERTENSION

In some patients, hypertension is known to be secondary to a specific disease process. Causes of secondary hypertension include renovascular abnormalities, renal parenchymal disease, primary hyperaldosteronism, Cushing's disease, adrenogenital syndromes, pheochromocytoma and certain drugs. In over 90% of adult hypertensive patients, however, there is no known cause of hypertension (Berglund, Anderssen and Wilhelmsen, 1976) and the disease must be classified as primary, benign, idiopathic or essential hypertension. Of course, hypertension is not really benign.

No single theory of the pathogenesis of essential hypertension has been generally accepted. Page (1949) recognized the fact that many systems participate in the regulation of arterial pressure and suggests that it is unlikely that moderate failure of one control system will lead to hypertension. Still, most workers in hypertension research have focused on one system at a time in an attempt to identify a pathogenic mechanism for hypertension. Several of the more popular theories used to explain human hypertension will be described. It will be seen that many of them are based on epidemiologic evidence and lack adequate experimental verification.

Genetic: It is not established to what extent the observed correlation between parental and offspring blood pressures is due to common heredity or a common environment (Tyroler, 1977). We are also ignorant concerning the basis for the observation that the incidence of hypertension in the Black population of the United States is 2 to 4.5 times

greater than that of the White population (Hypertension Detection and Follow-up Cooperative Group, 1977).

Dietary: Two dietary factors strongly correlate with hypertension. First, high salt intake both in individuals (Dahl and Love, 1954) and in cultures (Dahl, 1961b) is associated with hypertension. Salt is presumed to act to promote hypertension by either an absolute elevation of plasma volume or an inappropriately high volume relative to the blood pressure. Second, obesity was seen in 15.9% of 30 to 59 year old men with hypertension but in only 2.9% of a similar group of normotensive men (Kannel, Brand, Skinner et al., 1967). Weight change has been positively associated with blood pressure changes (Kannel and Sorlie, 1975) and hypertension has been effectively treated by caloric restriction (Reisin, Abel, Modan et al., 1978). The mechanisms responsible for the association between elevated body weight and hypertension are not defined.

Stress: Both acute (Brod, Fencl, Hejl et al., 1959; Lorimer, MacFarlane, Provan et al., 1971) and chronic (Graham, 1945; Cobb and Rose, 1973) mental stress were associated with elevated blood pressure. Evidence that environmental factors such as diet and stress are important in the progression of hypertension includes the observation in several primitive societies that blood pressure does not increase with age (Prior, Evans, Harvey et al, 1968;

Page, Danion and Moellering, 1974).

Hormonal: While hypertensive patients do not consistently have elevated plasma renin activity (PRA), several workers conclude that, in those patients with elevated PRA, renin has a pathogenetic role in the hypertension (Case, Wallace, Keim et al, 1977). Plasma nonrepinephrine was reported to be elevated in hypertension of short duration (Louis, Doyle and Anavekar, 1973). Deficiency of peripheral vasodilators such as prostaglandins of the A and E class is a possible contributing cause of hypertension that has received a great deal of attention recently. No clear correlation between blood pressure and plasma prostaglandin levels has been demonstrated (Lee, Patak and Mookerjee, 1976) but prostaglandins usually act locally and plasma levels may not indicate functional activity of the hormones.

Renal: It is known that humans with hypertension have an enhanced natriuretic response to sodium load (Lowenstein, Beranbaum, Chasis et al., 1970; Luft, Grim, Willis et al., 1976). Other renal abnormalities are more difficult to demonstrate directly. Although renal histology is normal in most patients with hypertension (Kincaid-Smith, 1975), there is reason to believe that renal function is abnormal in essential hypertension. In the next section I will describe some of the theoretical and experimental work

that has put renal function at the center of many analyses of the pathogenesis of hypertension.

Although it is apparent that research on the pathogenesis of hypertension is not limited to any one organ, the role of the kidney in hypertension will be the focus of the rest of this presentation.

THE ROLE OF THE KIDNEY IN HYPERTENSION

Historical

The association between hypertension and renal disease has been appreciated for over 150 years. In 1827, Richard Bright (1827) described a series of 23 patients with albuminous urine and anasarca. This condition became known as Bright's disease. In a later report he stated that a hard shrunken kidney at autopsy was often found with cardiac hypertrophy (Bright, 1836). He viewed the cardiac hypertrophy as being due either to an altered quality of the blood which directly stimulated the heart or as a compensation for the increased force required to propel blood through a periphery which had a higher resistance.

Several other early workers confirmed the relation between Bright's disease and a hard pulse or other properties associated with hypertension. Most of them thought that the renal disease occurred first and that in some manner the damaged kidney was responsible for the hypertension (Fahr, 1919). Mahomed (1879) questioned the cause-effect relationship between renal disease and high blood pressure, claiming that it was an alteration in the blood which causes the high blood pressure and that the high blood pressure itself will damage the kidney. There is still no resolution to the controversy stated by the question, "Does renal disease cause hypertension or is renal damage secondary to the hypertension?" In 1881 Leyden reached the prophetic conclusion that "...to the kidneys, among other things, also falls the function of acting in regulation of the aortic pressure" (cited in Gordon, 1977).

During the hundred years after Bright's reports there were some partially successful attempts to produce an experimental model of hyper-

tension by assaulting the kidney but it was Goldblatt, Lynch, Hansal et al. (1934) who produced the first reliable model of hypertension. By placing silver clamps around one or both renal arteries in the dog, Goldblatt was able to produce sustained hypertension which reached a plateau about 60 days after the clamps were applied. He did not at that time draw conclusions about the etiology of the renal hypertension. We must look to another line of research to formulate concepts that will aid us in understanding the pathogenesis of this and other forms of hypertension.

Salt and Hypertension

By describing the role of central venous pressure in the regulation of cardiac output (Starling, 1918) and the relation between arterial pressure and sodium excretion rate in the isolated kidney (Starling and Verney, 1925), Starling laid the basis for a volume-dependent, and therefore salt-dependent, theory of hypertension (Borst and Borst deGeus, 1963).

In agreement with Starling's concepts, one of the most consistent clinical observations is the efficacy of a strict low-salt diet in the treatment of hypertension. Ambard and Beaujard (1904) recommended a low-salt diet as therapy for hypertension. This was reiterated by Allen (1920) who recognized the difficulty of maintaining a strict low-salt diet but nonetheless emphasized the importance of diet in antihypertensive therapy. The value of a low-salt diet received convincing support by the studies of Kempner (1944; 1948) who used a rice-fruit diet which had a very low salt content to treat hypertension effectively in 62% of his hypertensive patients.

When Meneely studied the relation between the percentage of salt in the diet and systolic blood pressure in Sprague-Dawley rats, he found a strong positive correlation between these parameters at ages from 9 to 14 months (Meneely, Tucker, Darby et al., 1953). Complementary evidence of the hypertensinogenic properties of a high-salt diet in man is not as convincing.

In a four week study of normotensive young male prisoners on a moderate sodium load (5.6 $mEq \cdot kg^{-1} \cdot day^{-1}$), no relationship was found between the level of salt intake and the blood pressure (Kirkendall,

Connor, Abboud et al., 1976). An equally well-documented four week study of healthy young males used a sodium load of more than 9.5 mEq·kg^{-1}·day^{-1} (McDonough and Wilhelm, 1954). These workers found a significant 23% rise in both systolic and diastolic blood pressure as well as a 10% increase in the plasma volume with the sodium load. There is also a recent report of the blood pressure response in humans to a more extreme increase in sodium intake. Murray, Luft, Block et al. (1977) studied four normal adult males and found that, when sodium intake was increased from 10 to 1500 milliequivalents (mEq) per day, there was a significant concomitant increase in arterial pressure from 86 to 100 mmHg (Figure 4). If one assumes that the subjects in this study weighed 70 kg, their maximal sodium intake was 21.4 mEq·kg^{-1}·day^{-1}. The normal American dietary sodium intake is about 2.5 mEq·kg^{-1}·day^{-1}. Thus, it is apparently necessary to increase sodium intake as much as 4- to 9-fold in order to see a significant change in blood pressure over the course of a week or two. Baboons, studied for several years while on a high-sodium diet of 26 mEq·kg^{-1}·day^{-1}, developed a significant, irreversible hypertension (Cherchovich, Capek, Jefremova et al., 1976).

Thus, we have the impression from clinical and epidemiological studies that high salt intake on either an acute or chronic basis may be related to an elevated arterial pressure. Further, several reports have indicated that hypertension may be prevented or even reversed by a low-salt diet.

Integrating human epidemiological studies with the results from a rat model of experimental hypertension, Dahl developed an impressive set of data to buttress his assertion that the level of salt intake in a

group of rats or men is related to the incidence of hypertension. He found that the incidence of hypertension in a White American population was correlated with the self-reported level of salt consumption (Dahl and Love, 1954). When he examined the reports of salt intake from five cultures with various mean salt intakes from 4 to 26 grams per day, Dahl (1961) found a linear relation between salt intake and the prevalence of hypertension. An interesting study of death from stroke in Japan found a geographic coincidence of groups with high salt intake, high blood pressure and stroke (Sasaki, 1962). Workers in the South Pacific (Prior, Evans, Harvey et al., 1968; Page, Danion and Moellering, 1974) and in Africa (Shaper, 1972) found that even between groups that were genetically similar, low salt intake was associated with low to normal blood pressure, whereas the incidence of hypertension was much higher in those cultures in which salt intake was high. An interesting additional observation is that mean blood pressure did not increase with age in adults living in cultures accustomed to a low-salt diet. (Prior et al., 1968; Shaper, 1972; Page et al., 1974; Oliver, Cohen and Neel, 1975). This lends empirical support to the practice of defining the normal limits of blood pressure in absolute terms rather than using a correction for age (Hypertension Detection and Follow-up Program Cooperative Group, 1977).

A criticism of these epidemiological studies is that they cannot clearly assign cause and effect roles to salt intake and hypertension. The rat model of salt-sensitive hypertension developed by Dahl, Heine and Tassinari (1962) offers an opportunity to examine this problem more critically. This experimental model of hypertension was a natural extension

of the earlier work by Meneely (Meneely, Tucker and Darby, 1952; Meneely, Tucker, Darby et al., 1953; Meneely, Lemley-Stone and Darby, 1961).

Dahl selectively inbred two lines of rats from a Sprague-Dawley colony which either became hypertensive or remained normotensive on a high-salt diet (Dahl, Heine and Tassinari, 1962); the two strains were called sensitive and resistant respectively. After three generations, rats from the sensitive strain would remain normotensive until challenged with a high-salt diet after which they became clearly hypertensive. Rats from the resistant strain remained normotensive even on a high-salt diet. As have many others, Dahl emphasized the necessity of the presence of two or more hypertensinogenic factors simultaneously to produce hypertension. He asserts that the interplay of diet and heredity was responsible for hypertension in his salt-sensitive rats.

The positive correlation between salt intake and blood pressure is a general phenomenon which has been observed in several rat strains. Hall, Ayachi and Hall (1975a) studied the effects of high salt intake on blood pressure in four different types of rats. Sprague-Dawley rats from two sources, and Wistar rats became hypertensive when they were given normal saline to drink. Only "Fischer 344" rats remained normotensive, possibly because these rats limited their salt intake to normal amounts even with saline drinking water. Even the spontaneously hypertensive (SHR) rat, which becomes hypertensive on a normal salt diet, had a lower systolic blood pressure and increased longevity when placed on a low-salt diet (Dahl and Tuthill, 1974).

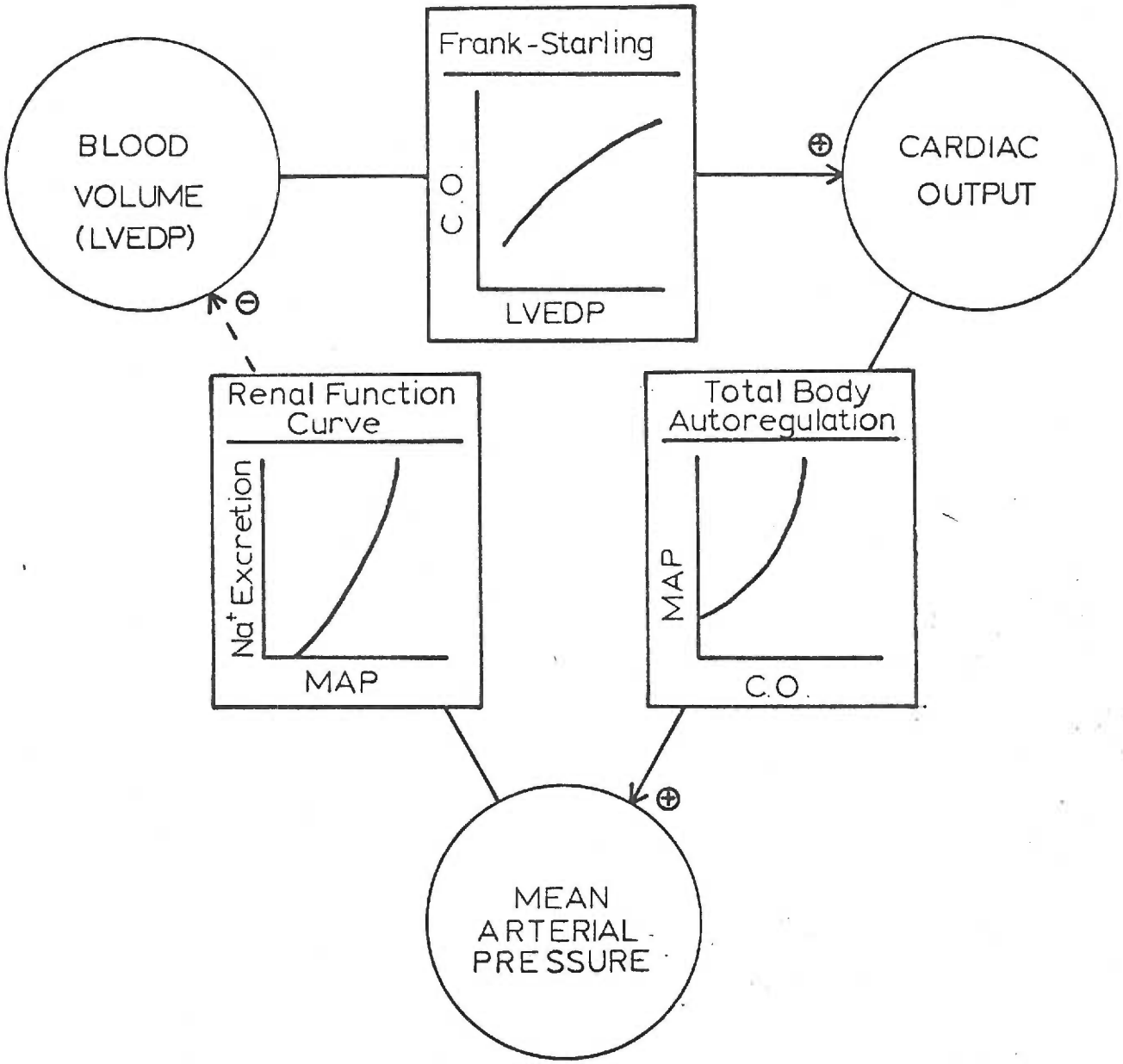
The Analysis of Guyton

Although the idea that renal function is central to the regulation of blood pressure has a long history, general appreciation and acceptance of the concept came only after the publication of a series of theoretical and empirical studies by Guyton beginning in 1963 (Langston, Guyton, Douglas et al. 1963) with the observation of the synergistic nature of high-salt diet and partial nephrectomy in producing hypertension in dogs.

Figure 1 summarizes the mechanisms by which the relation between renal sodium excretion rate and renal perfusion pressure may act to control chronic arterial pressure. Should an initial disturbance such as the infusion of norepinephrine increase the mean arterial pressure, this will lead to an increase in sodium excretion rate. If sodium intake remains unchanged, the blood volume will fall and, with a fall in venous return, cardiac output will also fall. A lower cardiac output will act to lower arterial pressure, tending to correct the initial hypertensive disturbance in this example.

Let us now examine this system in more detail and see why Guyton arrived at the conclusion that in all cases of long-standing hypertension there must be an abnormality in the relationship between sodium excretion rate and arterial pressure (Guyton, Coleman, Cowley et al., 1974a). In expanding on Borst's thesis, Guyton used analytical models based on control theory, believing that "...the analytical approach to understanding function of the bodily mechanisms can lead to far greater depths of meaning than can possibly be true when using the informal, intuitive approach" (Guyton, Coleman, Cowley et al., 1972). Using a computer model of the

Figure 1: Diagramatic summary of the systems involved in the regulation of arterial pressure. Refer to the text for a full description of the system.


BLOOD VOLUME (LVEDP)
Frank-Starling
C.O.
LVEDP
CARDIAC OUTPUT
Renal Function Curve
Na+ Excretion
MAP
Total Body Autoregulation
MAP
C.O.
MEAN ARTERIAL PRESSURE

systems which regulate arterial pressure, he demonstrated that, in the long run, the most powerful system which regulates blood pressure is the salt and water excretion system. Guyton cautions that specific results from computer simulations are of questionable specific validity since the experimental values for many parameters which must be entered into the program are not known with sufficient precision. Further, it is not really valid to use linear control theory in the analysis of non-linear biological systems. The conclusion that the renal handling of sodium and water is of overriding importance in long-term pressure regulation may not, however, suffer from these criticisms since the conclusion does not depend upon specific experimental values or the linear nature of the model. This conclusion will now be examined in some detail.

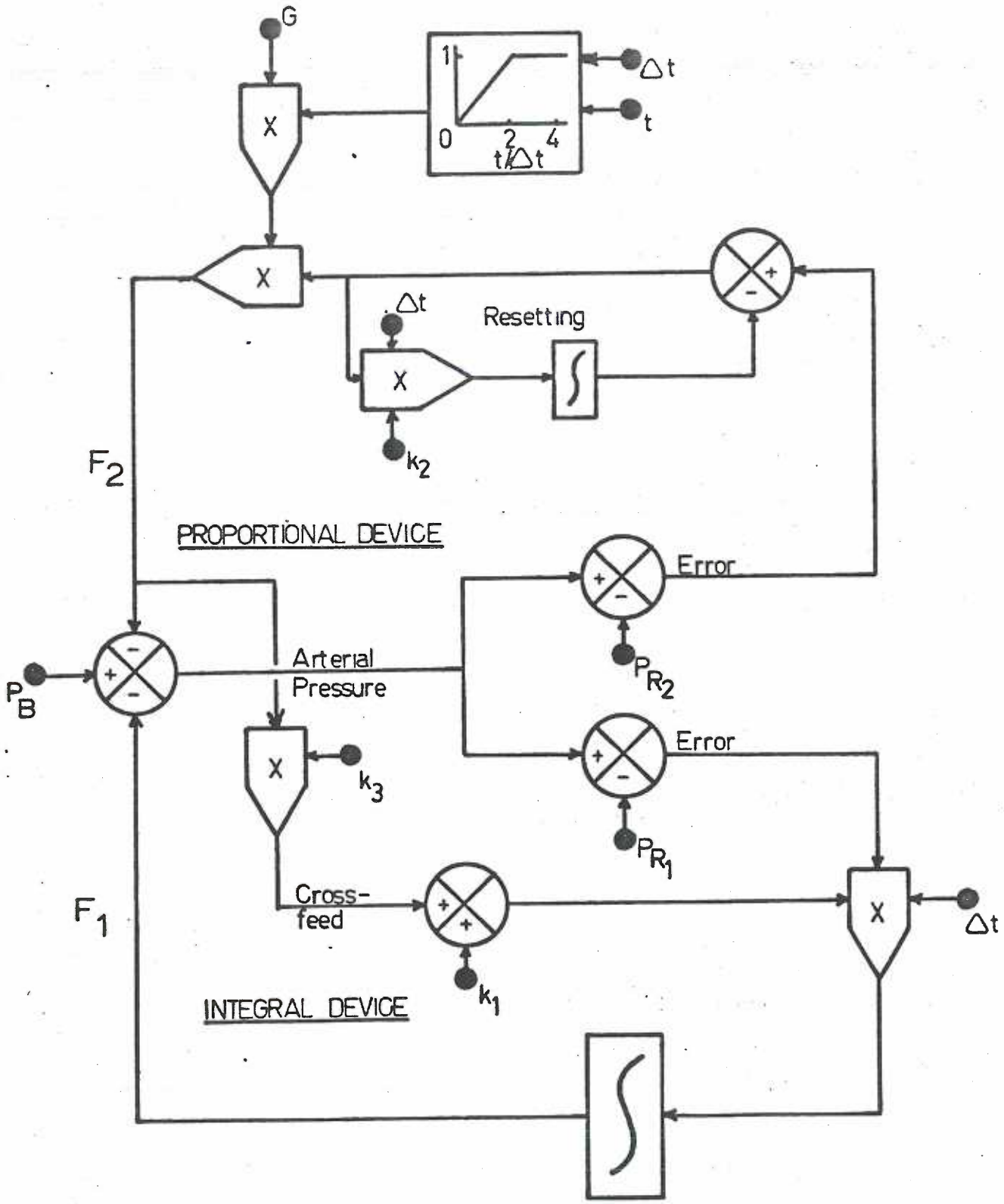
Two classes of control systems, proportional and integral, will be used here to examine the nature of arterial blood pressure control. Many of the devices concerned with blood pressure regulation may be modeled as proportional devices. This type of device will act to minimize deviations of the controlled variable from a reference value. The nature of the reference pressure in the blood pressure regulation system is not at all clear. It may be useful to think of a normal pressure rather than a reference pressure. Pressure may be sensed either directly or, more likely, by some other parameter that is related to pressure, such as arterial wall strain. In a control device, the error is the difference between the normal and the actual pressure value. The error is related to the magnitude of the corrective response by the system. The quotient of the output (corrective response) divided by the input (error) is known as the gain of the system. The error will be small when the gain is

large but there will always be some finite error with a purely proportional device.

The baroreceptor is the sensor for a well known blood pressure regulating system which acts like a proportional system. The output of the baroreceptor is related to the difference between the actual pressure and the normal pressure, which is the error. A proportional device which could represent the baroreceptor is shown in analog form at the top of Figure 2. Included in the design is an allowance for gradual onset of the baroreceptor system response and a means to reset the reference point of the device (Guyton, Coleman, Cowley et al., 1974a). It is known that there is a resetting of the baroreceptor in rats over a period of days for downward changes in the prevailing pressure (Salgado and Krieger, 1978) and within hours for upward changes (Salgado and Krieger, 1973). After complete baroreceptor resetting subsequent to either an increase or decrease in the prevailing pressure, the threshold pressure for aortic nerve activity is raised or lowered respectively. Although the prevailing blood pressure may be changed, aortic nerve activity after complete resetting is not different from what it was before blood pressure was altered.

In the case of an integral device, the magnitude of the correction is not related only to the value of the error; it is related to the time integral of the error. The renal salt and water excretion system is the only recognized example of an integral device among the systems which regulate arterial pressure. The kidney regulates blood pressure by controlling salt and fluid excretion rate and its time integral, namely the body fluid volume. If blood pressure is higher than the normal pressure,

Figure 2: An analog-style representation of a proportional control device (e.g. baroreceptor system) and an integral device (i.e. renal fluid excretion system) and their points of interaction in the regulation of arterial pressure (Adapted from Guyton, Coleman, Cowley et al. 1974a). Symbols: t= total time elapsed since a change in basic pressure, Δt= time increment interval (fixed as 1/200th of the total simulation time), P_B= basic pressure without the intervention of either control device, P_{R1}= reference pressure for the integral device, P_{R2} = reference pressure for the proportional device, F_1 = feedback from the integral device, F_2= feedback from the proportional device, G = gain of the proportional device, k_1 = constant which relates the error of the integral device to the rate of change in that device's influence on arterial pressure, k_2 = constant controlling the rate of resetting of the proportional device, k_3 = constant governing the amount of cross-feed from the proportional device to the integral device.


G
X
1
0
2
4
t/Δt
Δt
t
X
Δt
Resetting
X
k2
F2
PROPORTIONAL DEVICE
Error
PB
Arterial
Pressure
PR2
X
k3
Error
PR1
F1
Cross-
feed
X
Δt
k1
INTEGRAL DEVICE

fluid loss will exceed intake so that there is a net loss of body fluid. As long as there is any discrepancy between actual and normal pressure, fluid loss will continue and the fluid volume of the body will decrease. Given enough time, the difference between actual and normal pressure, which is the error, will be reduced to zero. This means that, in time, the gain will be infinite. This feature leads us to conclude that renal salt and water excretion mechanisms probably dominate the long-term control of arterial blood pressure.

An integral device is represented on the bottom half of Figure 2. Included is a cross-feed from the proportional or baroreceptor system since the vascular changes brought about by baroreceptor activation affect not only the peripheral vasculature but also the renal vascular resistance and the rate of fluid loss.

The behavior of a system with both a proportional device and an integral device can best be shown by some analytical examples. The representation in Figure 2 was translated into a series of equations which were solved by numerical methods on an HP-9810 calculator with plotter. Refer to Figure 2 for the meaning of the symbols used in the following analytic equations.

The feedback from the two devices can be analyzed separately by an iterative process starting from time t=0 and using increments of Δt. At time t=0, $F_1=F_2=0$.

The feedback from the proportional device will be:

$$F_2(t+\Delta t) = G\Big\{ P_B - F_2(t) - F_1(t) - P_{R_2} - \{[(P_B - F_2(t) - F_1(t) - P_{R_2})k_2\,\Delta t]_{t=0}$$

$$+[(P_B - F_2(t) - F_1(t) - P_{R_2})k_2\,\Delta t]_{t=\Delta t} + \cdots$$

$$+[(P_B - F_2(t) - F_1(t) - P_{R_2})k_2\,\Delta t]_{t=t}\}\Big\}$$

Where $F_2(t+\Delta t)$ is the value of F_2 at time $t+\Delta t$.

The feedback from the integral device will be:

$$F_1(t+\Delta t) = \{(P_B - F_2(t) - F_1(t) - P_{R_1})\,(F_2(t)k_3 + k_1)\,\Delta t\}_{t=0}$$

$$+\{(P_B - F_2(t) - F_1(t) - P_{R_1})\,(F_2(t)k_3 + k_1)\,\Delta t\}_{t=\Delta t} + \cdots$$

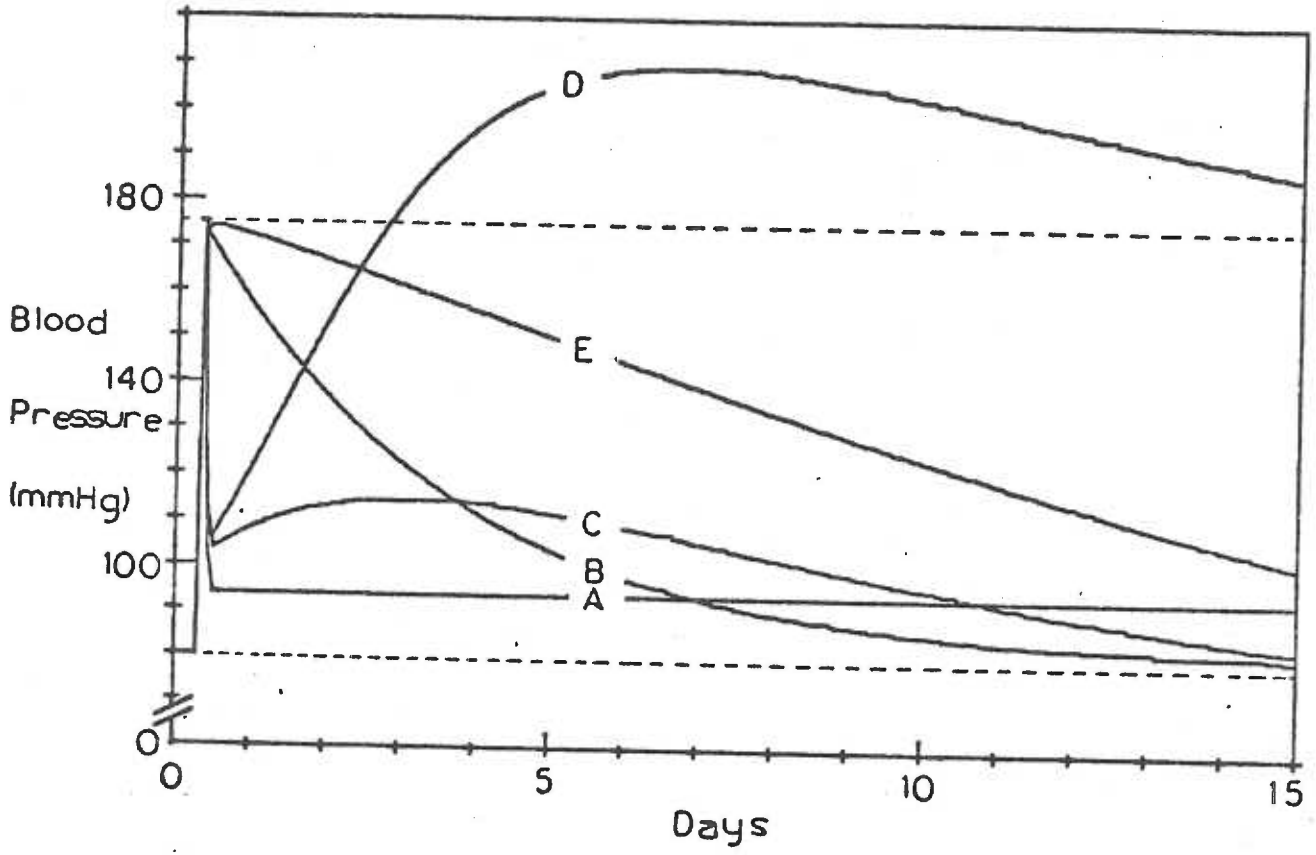
$$+\{(P_B - F_2(t) - F_1(t) - P_{R_1})\,(F_2(t)k_3 + k_1)\,\Delta t\}_{t=t}$$

The arterial pressure at any time t (MAP(t)) is given by:

$$MAP(t) = P_B - F_1(t) - F_2(t)$$

Values for the parameters were chosen so that the renal salt and water system compensated for a simulated volume load in a week or two. Baroreceptors were activated in much less than a day and reset in about a day (Guyton, Coleman, Cowley et al., 1972). The solutions of these equations for several sets of parameters are shown in Figure 3. Each

Figure 3: Temporal changes in blood pressure produced by a sudden increase in the basal blood pressure (P_B) from 80 to 175 mmHg. Values listed in Table 1 were used in the model shown in Figure 2.


D
180
Blood
E
140
Pressure
(mmHg)
C
B
100
A
0
0
5
10
15
Days

curve is a simulation of the response of all or part of the system in Figure 2 to an acute volume load which, if uncorrected, would raise mean arterial pressure from 80 to 175 mmHg.

Table 1: Values of the parameters used in computing the five curves in Figure 3. See Figure 2 for the meaning of the parameters.

Curve	P_B	P_{R_1}	P_{R_2}	G	k_1	k_2	k_3
A	175	80	80	6	0	0	0
B	175	80	80	0	0.3	0	0
C	175	80	80	6	0.3	1.5	0.01
D	175	175	80	6	0.3	1.5	0.01
E	175	80	175	6	0.3	1.5	0.01

Curve A of Figure 3 shows the effect of a non-resetting baroreceptor system acting alone. The effect of the renal salt and water excretion system in isolation is shown in curve B. Note that, although the renal system responds more slowly than does the baroreceptor system, the error of the renal system approaches zero within two weeks. The results of acute studies of the combined system will be dominated by the baroreceptor system, obscuring the long-term importance of the renal system. With both the proportional and the integral device operating and with resetting of the proportional device and a cross-feed from the proportional to the integral device, the system responds in a manner depicted in curve C. By reducing the duration of pressure elevation and the consequent diuresis, the baroreceptor system actually causes the renal system to bring the pressure back down more slowly than it would have in the absence of the baroreceptor system.

Previously, I have assumed that the reference pressure for both devices was the same (80 mmHg). Let us now consider the effect of raising the reference pressure of only one device coincident with the acute volume load. Should the renal reference pressure be raised to 175 mmHg as in curve D, the actual pressure will eventually rise to this value. Raising the reference pressure of the baroreceptor system to 175 mmHg while keeping the renal reference pressure at 80 mmHg (curve E) does not, in the long-term, raise the actual pressure above 80 mmHg. The last two examples provide particularly clear examples of the long-term dominance of the integral device.

Because the fluid volume system is a type of integral control device with infinite long-term gain, it far surpasses the strength of any neural, humoral or structual vascular components of blood pressure regulation since those systems which do not operate through the renal fluid control system have finite gains. Adapting the arguments of Ledingham (1964), Coleman and Guyton (1969) modified this basic idea to agree with clinical data which indicate that there may be a normal or even diminished blood volume in established hypertension (Hansen, 1968; Dustan, Tarazi and Bravo, 1972; Safar, Chau, Weiss et al., 1976) and that cardiac output may be normal (Frohlich, Tarazi and Dustan, 1969). Guyton proposed that, in the early stages of hypertension, there is an elevated extracellular fluid volume and increased cardiac output. He suggested that the body responds to the resulting tissue hyperperfusion with an increase in vascular resistance. This secondary increase in total vascular resistance obscures the hypervolemic origins of the hypertension. By this hypothesis, the increase in blood volume and cardiac output that was

present during the early stages of hypertension is replaced by an increase in total peripheral resistance which operates to return local tissue perfusion levels to normal and maintains the hypertension at a normal cardiac output and normal plasma volume. The increase in total peripheral resistance means that, even though plasma volume is normal or even lower than normal in established hypertension, plasma volume is inappropriately elevated in view of the normal inverse relation between plasma volume and arterial pressure (Safar, Chau, Weiss et al., 1976). It is the sum of these resistance changes which Guyton (Coleman, Granger and Guyton, 1971) terms "whole body autoregulation" of tissue perfusion.

Guyton's studies, the well-known efficacy of diuretics and of low-salt diets in the treatment of hypertension, the hypertensinogenic effect of a high-salt diet and the association of renal abnormalities with secondary hypertension all indicate that the kidney is importantly involved in conditions in which there is a chronic elevation of arterial pressure. This is not to say that the kidney itself is abnormal in all cases of hypertension. It is possible that the primary pathology in a given type of hypertension is extrarenal. The point of Guyton's analysis is that, for chronic hypertension to persist, this extrarenal abnormality must be reflected in an abnormal ability of the kidney to excrete salt and water:

> "... almost no effect can cause continued elevation of arterial pressure unless it in some manner affects the kidneys."
> (Guyton and Coleman 1969)

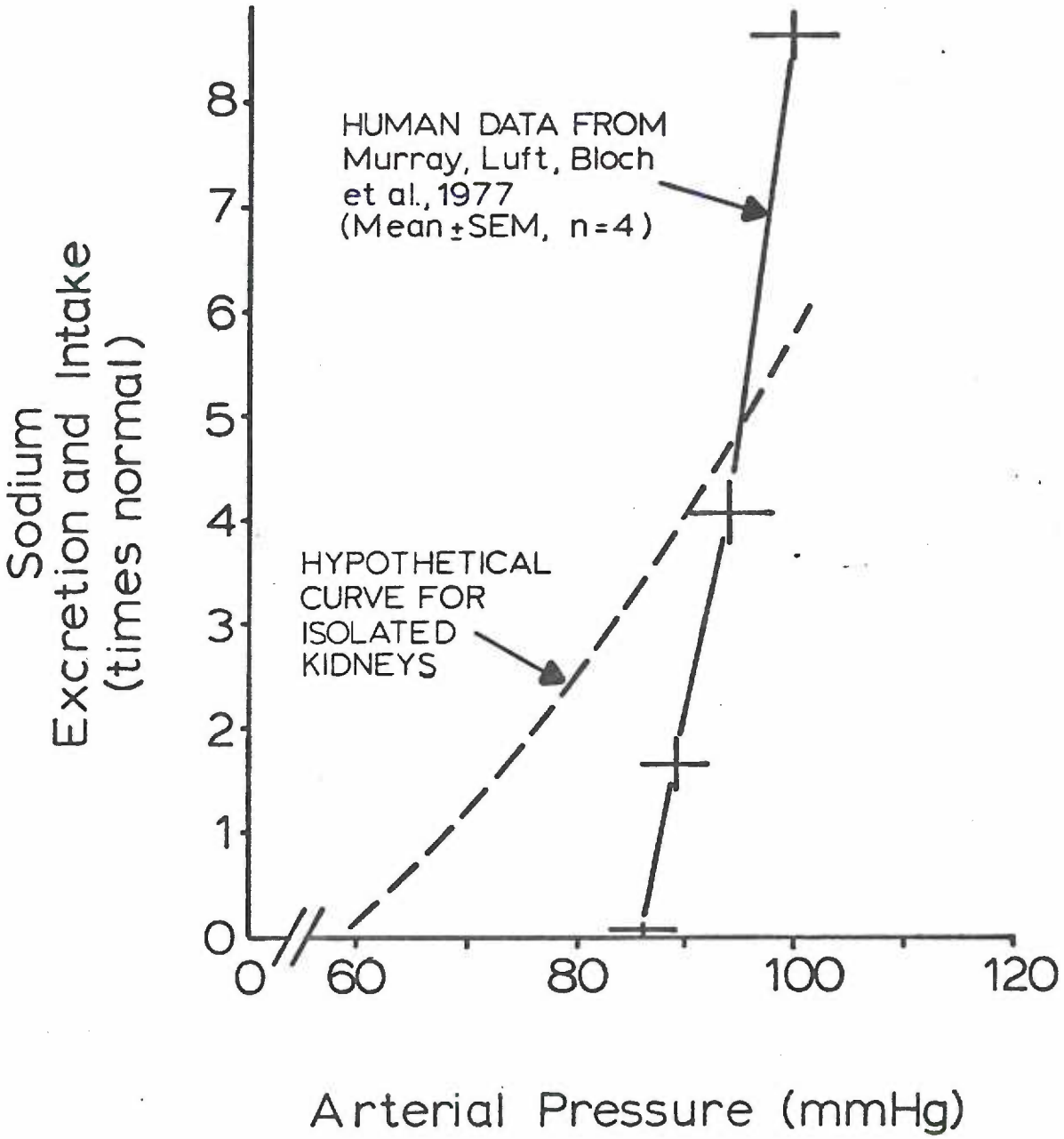
This analysis is in agreement with clinical experiences concerning the antihypertensive efficacy of drugs which act at extrarenal sites, including the central nervous system and the autonomic nervous system. In

dogs the observable hemodynamic consequences of volume loads (Vogel, 1966; Coleman and Guyton, 1969; Granger and Guyton, 1969; Guyton, Coleman, Cowley et al., 1972) support this analysis.

At the center of Guyton's analysis of the regulation of arterial blood pressure (Guyton, Coleman and Granger, 1972; Guyton, Coleman, Cowley et al., 1972; Guyton, Coleman, Cowley et al., 1974a) is the relation between the sodium excretion rate and the arterial pressure which he calls the renal function curve. The solid line in Figure 4 is an example of a renal function curve which was redrawn from the data of Murray, Luft, Block et al. (1977) obtained with four normal human subjects, each at four levels of sodium intake. In assessing their data, I assumed that 10 g of salt (172 milliequivalents of sodium) is found in the normal daily American diet. These data provide a recent example of the relation between sodium excretion rate and blood pressure which has been recognized for many years. As I shall explain, not only can increased salt intake lead to an elevated arterial pressure as in the study of Murray et al. (1977) but an increase in blood pressure will tend to increase sodium excretion rate.

Fifty years ago Starling and Verney (1925), working with isolated dog kidneys, established the correlation represented by the renal function curve and stated that "... blood pressure as a factor in the rate of urine elimination may, therefore, be taken as established." Growth, evaporation and fecal losses account for a rather constant if not minor difference between intake and renal excretion of salt and water so that, in the chronic state, intake and renal excretion of salt and water are closely related.

Figure 4: The renal function curve. Arterial pressure is related to sodium excretion rate. In the chronic state, sodium intake and excretion will be the same. The observed renal function curve for normotensive humans (solid line) is from Murray, Luft, Block et al. (1977). The curve for isolated human kidneys (dashed line) is based on extrapolation from reports using isolated animal kidneys. There are no reports of the renal function curve in isolated human kidneys.


Sodium
Excretion and Intake
(times normal)
8
7
6
5
4
3
2
1
0
0
60
80
100
120
HUMAN DATA FROM
Murray, Luft, Bloch
et al., 1977
(Mean ± SEM, n=4)
HYPOTHETICAL
CURVE FOR
ISOLATED
KIDNEYS
Arterial Pressure (mmHg)

A feature of feedback systems is that we cannot always use the concept of dependent and independent variable (Riggs, 1963). If we open the feedback loop for the renal sodium excretion and volume regulation system by studying the isolated kidney it is appropriate to consider the renal function curve in terms of an independent variable (mean arterial pressure) and a dependent variable (sodium excretion rate). If the feedback loop is closed, however, the variables are interdependent. In the intact preparation, we may vary the sodium excretion rate acutely by changing the blood pressure or we may vary the mean arterial pressure by changing the sodium intake which is equivalent to changing the long-term sodium excretion rate. As long as the feedback loop is not interrupted, variables can then be spoken of as being interdependent.

Recognizing that the tradition of representing the independent variable on the abscissa and the dependent variable on the ordinate is not relevant, I will use the convention that sodium excretion rate or the related water loss is presented on the ordinate and that mean arterial pressure is presented on the abscissa. With this orientation, a rightward shift of the renal function curve, as in hyperaldosteronism or a major loss of renal mass, will mean that an elevated arterial pressure is required to maintain a balance between sodium excretion rate and a normal sodium intake level.

An important characteristic of the renal function curve in vivo is its steep slope. In normal people a large increase in salt intake is associated with only a minor change in arterial pressure (Figure 4). The nature of the renal function curve is quite different depending on whether the curve is measured in the isolated kidney (McDonald and

de Wardener, 1965; Tobian, Johnson, Lange et al., 1975; Thompson and Dickinson, 1973; 1976), the intact animal with acute renal arterial pressure changes (Selkurt, Hall and Spencer, 1949; Selkurt, 1951; Blake, Wegria, Ward et al., 1950; Thompson and Pitts, 1952; Beierwaltes and Arendshorst, 1978), the intact salt-loaded animal with consequent chronic differences in blood pressure (Bianchi, Baer, Fox et al., 1975; Hall, Ayachi and Hall, 1975a; Berglund, Wikstrand, Wallentin et al., 1976; Cherchovich, Capek, Jefremova et al., 1976; Murray, Luft, Bloch et al., 1977; Norman, Enobakhare, DeClue et al., 1978; Pan, Young and Guyton, 1978) or the intact human with acute ganglionic blockade (Birkenhager, van Eps and de Vries, 1962).

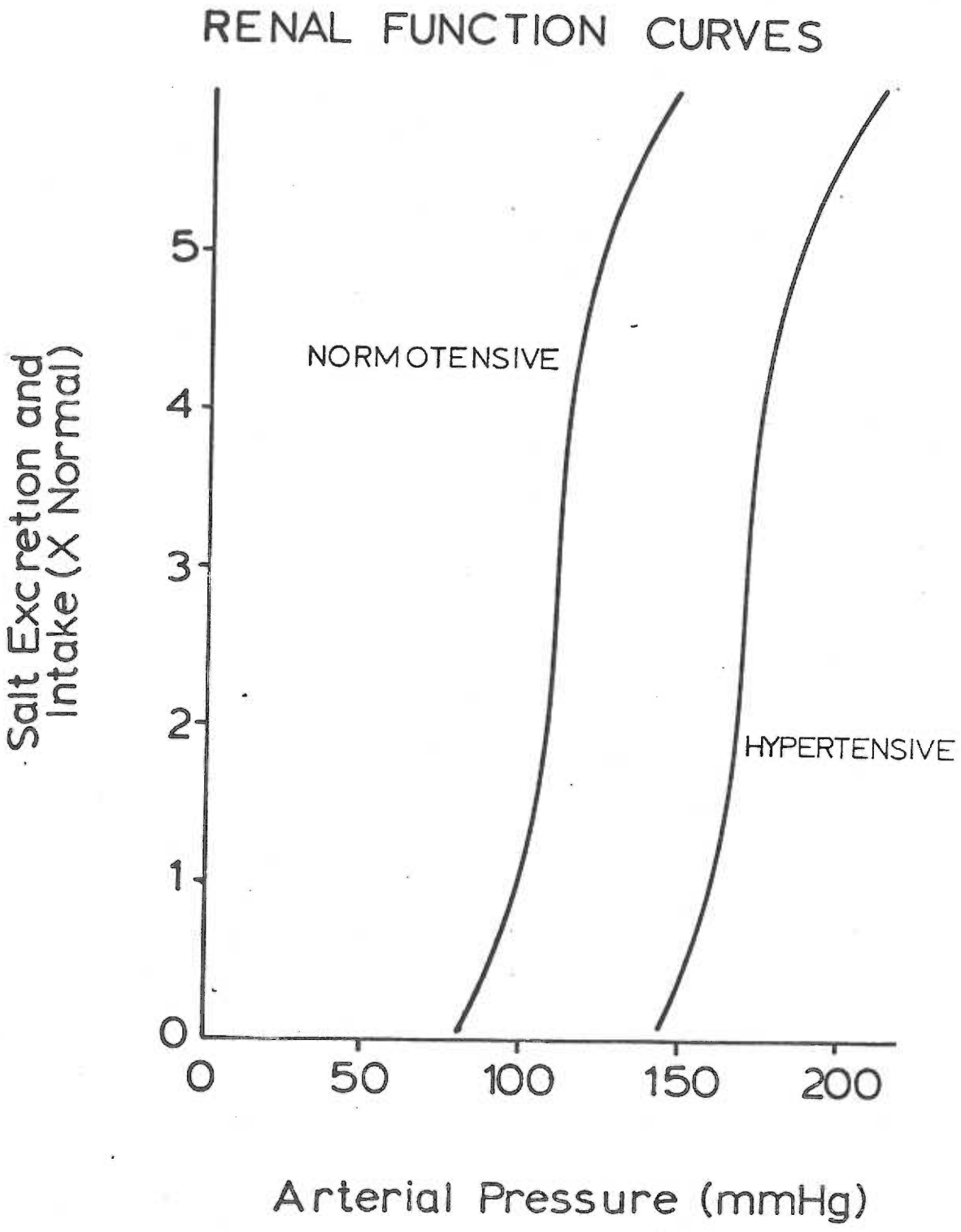
In general, the in vivo renal function curves are steeper than curves for isolated kidneys because of extrarenal influences on sodium excretion. Human kidneys, however, are not readily available for isolated experiments and we must rely on animal studies to establish the probable nature of the renal function curves for isolated human kidneys. In Figure 4, the dashed line is a hypothetical representation of the renal function curve for isolated human kidneys. There are extrarenal influences on the intact kidney, such as neural and vascular tone, circulating renin, aldosterone, prostaglandins and vasopressin, which act to change sodium excretion at a given mean arterial pressure. The net effect of these varying extrarenal influences implies that the intact renal function curve is steeper than the curve for isolated kidneys.

It has not been established whether salt intake in hypertensive patients is greater than or less than in normotensive individuals. Using interviews of a general population to assess dietary salt intake, Dahl and

Love (1954) found that 10.1% of those who added salt to all foods before tasting them had hypertension whereas only 1% of those who never used added salt had hypertension. In a large study of untreated men with essential hypertension, Berglund, Wikstrand, Wallentin et al. (1976) found that there was a significant negative correlation between blood pressure and voluntary sodium intake as estimated from urine collections. This correlation was positive in normotensive individuals. It appears that high salt intake may have some role in initiating hypertension but that hypertensive patients restrict their intake voluntarily. Berglund's patients were not treated for their hypertension but they had been informed of the diagnosis of hypertension and may have then modified their salt intake to conform with lay knowledge of the value of a low-salt diet in hypertension. Even if salt intake is moderately elevated in hypertension, this cannot by itself account for the severity of hypertension in view of the steep renal function curve in vivo. Consequently, the renal function curve in vivo must be to the right of the normotensive curve in most human and experimental hypertension (Figure 5).

In my presentation of the renal function curve, I will only present data for sodium intake or excretion. It should be realized, however, that the importance of an elevated sodium intake is that there will be a concomitant increase in body fluid volume. Guyton (Norman, Coleman, Wiley et al., 1975) studied nephrectomized sheep in which fluid volume was varied with hemodialysis. When plasma sodium concentration was then increased with deoxycorticosterone acetate (DOCA), only those sheep with increased extracellular fluid volume became hypertensive. Increased plasma sodium concentration was hypertensinogenic only when it was

Figure 5: Renal function curves for normotensive and hypertensive humans. These curves are based on theoretical considerations and are designed to illustrate the principle that the chronic renal function curve for the hypertensive patient will be shifted to the right of the normotensive curve. This shift need not be parallel.


RENAL FUNCTION CURVES
Salt Excretion and Intake (X Normal)
0
1
2
3
4
5
NORMOTENSIVE
HYPERTENSIVE
0
50
100
150
200
Arterial Pressure (mmHg)

accompanied by elevated extracellular fluid volume.

To point out that there must be a rightward shift of the in vivo renal function curve[1] is not to say that the kidney itself is abnormal in chronic hypertension. Table 2 is a partial list of mechanisms which may modify the relation between arterial pressure and sodium excretion in rats so that the renal function curve is shifted to the right. Table 2 is divided into those mechanisms which may modify sodium excretion rate by acting before the glomerular capillary barrier, those which may act at the glomerular capillary barrier and those mechanisms which may act on renal tubular structures. I will provide more detailed descriptions of some key mechanisms later when I discuss the pathogenesis of hypertension in the spontaneously hypertensive (SHR) rat. Examination of the pathogenesis of hypertension should not consider the factors in Table 2 or other factors in isolation but must consider how the factors can maintain the rightward shift of the renal function curve in hypertension.

[1] The phrase "rightward shift of the renal function curve" will be used here to mean that the renal function curve is to the right of the appropriate control renal function curve. By using the word "shift", I do not mean to imply anything about the prior position of the renal function curve.

Table 2: Physiologic and pathophysiologic mechanisms which may be capable of moving the position of the renal function curve of rats to the right.

Decreased Glomerular Capillary Filtration Pressure or Decreased Glomerular Capillary Plasma Flow at a Normal Renal Perfusion Pressure

- Increased stimulation of the renal sympathetic nerves
- Increased renal vascular or juxtaglomerular cell reactivity to sympathetic nerve stimulation
- Increased plasma colloid osmotic pressure
- Increased local or circulating angiotensin II concentration
- Increased local or circulating prostaglandin (PGE_2) concentration
- Increased plasma calcium concentration
- Structurally-based increase in pre-glomerular or post-glomerular renal vascular resistance

Decreased Glomerular Capillary Filtration Coefficient

- Increased local or circulating angiotensin II concentration
- Increased plasma calcium concentration
- Decreased renal mass

Increased Tubular Sodium Reabsorption

- Increased plasma colloid osmotic pressure
- Increased circulating mineralocorticoid concentration
- Increased stimulation of the renal sympathetic nerves
- Increased circulating vasopressin concentration
- Decreased natriuretic hormone concentration
- Increased local or circulating prostaglandin (PGE_2) concentration

THE SPONTANEOUSLY HYPERTENSIVE (SHR) RAT MODEL

The Value of Animal Models

The lack of a consistent hemodynamic, hormonal or environmental pattern in humans with essential hypertension constitutes an impediment to research on the pathogenesis of hypertension. Studies of hypertension in humans require large numbers of subjects in order to allow for the multitude of parameters which are related to hypertension but that cannot be controlled and must, therefore, be included in the analysis of results (Veterans' Administration Cooperative Study Group, 1967; 1970; 1972; Berglund, Andersson and Wilhemsen, 1976; Hypertension Detection and Follow-up Program Cooperative Group, 1977; Perry, Schnaper, Fitz et al., 1977; Medical Research Council Working Party, 1977). An understanding of many aspects of human hypertension requires long periods of prospective study since the disease usually progresses slowly with approximately 15 years between the onset of hypertension and the appearance of complications (Perera, 1955). In addition, much of the data on the pathologic changes during the development of hypertension, the behavior of isolated organs and the effect of extreme experimental manipulations cannot be obtained from any human study.

For these reasons, researchers have sought an animal model of human hypertension. Goldblatt's model of hypertension secondary to renal artery stenosis was an important advance but is clearly not directly applicable to most cases of human hypertension since severe renal vascular disease is usually not present. Work with several strains of rats with an inherited predisposition for hypertension which have been developed over the last 20 years promises to help unravel the processes which are relevant to human essential hypertension.

Development of Rat Strains with an Inherited Predisposition for Hypertension

During the 1950's, Sir Horace Smirk, working in Dunedin, New Zealand, developed an inbred strain of hypertensive rats (Smirk and Hall, 1958). Systolic blood pressures reached a plateau of 165 to 175 mmHg by 6 to 8 weeks in some rats. Unfortunately, only about 30% of the inbred strain would develop hypertension. This limits the usefulness of this animal model. The partial arrest of the development of hypertension with 6-hydroxy dopamine injections may indicate a role for the sympathetic nervous system in the pathogenesis of hypertension in this strain (Clark, 1971).

By 1962, Dahl, Heine and Tassinari (1962) had separated strains of Sprague-Dawley rats at Brookhaven which were either sensitive or resistant to the hypertensinogenic properties of dietary salt. Cross-transplantation studies indicated that the kidney may be involved in the pathogenesis of hypertension in the salt-sensitive rats (Dahl, Heine and Thompson, 1974).

Between 1964 and 1968, Bianchi developed a strain of hypertensive rats from a Wistar colony in Milan. As with the Dahl strain of hypertensive rats, there is strong evidence from transplantation studies to implicate the kidney directly in the pathogenesis of hypertension in this strain (Bianchi, Fox, DiFrancesco et al., 1973).

The rat that is now most widely used as a model of hypertension is the spontaneously hypertensive (SHR) rat which was developed in Kyoto by Okamoto and Aoki from 1960 to 1962 (Okamoto and Aoki, 1963). The SHR rat was derived by selective out-breeding of those animals with the highest

systolic blood pressure from a colony of Wistar rats. The most appropriate single normotensive control for the SHR rat is the Wistar-Kyoto (WKY) rat which was bred from the normotensive members of the Wistar colony at Kyoto.[1]

Mean arterial pressure of SHR rats was observed to be significantly elevated relative to WKY rats at 21 days (Lais, Rios, Boutelle et al., 1977). Practically all SHR rats become hypertensive with systolic blood pressures plateauing at about 190 mmHg and mean arterial pressures of about 150 mmHg. Both SHR and WKY strains are maintained by brother-sister mating at Kyoto, other research centers throughout the world and several commercial animal suppliers.

[1] The accepted use of the terms "SHR" and "WKY" is as adjectives rather than nouns (Institute of Laboratory Animal Resources, 1976).

Comparison of SHR Rats and Humans with Essential Hypertension

An important feature of the SHR rat and other major rat models of hypertension is the dominance of heredity as a factor in their hypertension. There are several studies which also support an important role for heredity in human hypertension. In these studies heredity was reported to account for 33% to 63% of the variability in blood pressure between parents and their children (Platt, 1959; Miall and Oldham, 1963; Pickering, 1968). The results of a recent study in Detroit, which used a family set method, suggest that heredity may be minor relative to environmental factors in predicting hypertension (Ranjit, Schull, Harburg et al., 1977). A difference in the degree of heritability of hypertension in humans and in the SHR rat would present a major problem in our ability to apply findings in SHR rats to humans with essential hypertension. This reservation aside, there are several features of the SHR rat which make it a valuable model for human hypertension.

Just as humans with hypertension live shorter lives than do normotensive individuals (Holme and Waaler, 1976), the median life-span of untreated SHR rats is 18 months compared to 24 months for WKY rats. (Nagaoka, Kikuchi, Kawaji et al., 1972). As in most cases of human hypertension, SHR rats develop hypertension spontaneously without any special treatment or diet. Most forms of human hypertension can be treated effectively with salt restriction. Likewise, a low-salt diet also offers significant protection against hypertension in SHR rats (Dahl and Tuthill, 1974). Some of the same antihypertensive drugs that are effective in the treatment of human hypertension are partially effective in the SHR rat; antihypertensive drugs will lower blood pressure and

eliminate organ damage (Freis, Ragan, Pillsbury et al., 1972; Folkow, Hallback, Lundgren et al., 1972) as well as increase longevity (Freis and Ragan, 1975). SHR rats did not, however, respond to some antihypertensive drugs (e.g. reserpine) which are effective in hypertensive humans (Frohlich and Pfeffer, 1975; Freis and Ragan, 1976).

A number of studies concerning the hemodynamic characterization of the adult SHR rat seem to indicate that the SHR rat is a suitable model for established human essential hypertension. Table 3 summarizes these similarities. Relevant parameters that are similar both in established human hypertension and in the SHR rat include an elevated heart rate, a normal blood volume, elevated total peripheral resistance, normal cardiac output and normal plasma dopamine beta hydroxylase activity. On the other hand, hypertensive humans tend to be overweight whereas the SHR rat is, if anything, underweight compared to the WKY rat.

Table 3: Comparison of various parameters in SHR rats and humans with essential hypertension. Young SHR rats are less than 20 weeks old. Data are given as mean ± SEM.

*$P < 0.05$ for the comparison of SHR vs. WKY rats or hypertensive vs. normotensive humans.

TABLE 3

	Humans			Rats		
		Hypertensive			SHR	
	Normotensive	Labile	Established	WKY	Young	Adult
Heart rate (min^{-1})	68 ±2 [1]	75±4 [1]	76 ±2* [1]	410±9 [2]	453±9 [2]	
				397±9 [3]		438±7* [3]
Red blood cells ($10^6 \cdot mm^{-3}$)				7.7±0.9 [4]		9.8±1.2* [4]
Hematocrit (%)				49.2±3.3 [5]		50.9±3.4* [5]
Plasma volume ($ml \cdot 100g^{-1}$)	4.42±0.01 [18]		4.11±0.01* [18]	4.9±0.1 [2]	5.0±0.1 [2]	
Blood volume ($ml \cdot 100g^{-1}$)	6.57±0.73 [6]		6.71±0.81 [6]			
Total body water ($ml \cdot 100g^{-1}$)				73.2±0.4 [2]	74.6±0.3* [2]	
Total peripheral resist. ($mmHg \cdot ml^{-1} \cdot min^{-1}$)	0.017±0.001 [1]	0.017±0.001 [1]	0.029±0.001* [1]			
($mmHg \cdot ml^{-1} \cdot min^{-1} \ kg^{-1}$)				0.247±0.017 [3]		0.388±0.014* [3]
Cardiac Index ($ml \cdot min^{-1} \cdot m^{-2}$)	3048±92 [1]	3399±145* [1]	2905±79 [1]			
($ml \cdot min^{-1} \cdot kg^{-1}$)				278±15 [3]		286±6 [3]
Weight (kg)				0.221±0.007 [3]	0.210±0.009 [3]	
				0.677±0.050 [2]		0.571±0.051 [2]
Percent obese	2.9 [7]	9.1* [7]	15.9* [7]			
Mean Arterial Pressure (mmHg)	93±1.6 [1]	106±2.3* [1]	130±3.5* [1]	89±1 [2]	109±3* [2]	
				110±3 [3]		154±2* [3]
Renal blood flow (% C.O.)				13.4±0.5 [8]	13.7±0.9 [8]	
				19.0±1.5 [9]		21.0±0.9 [9]

TABLE 3 (Continued)

	Humans			Rats		
		Hypertensive			SHR	
	Normotensive	Labile	Established	WKY	Young	Adult
Renal Blood Flow ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	1577 ± 97 [10]		1345 ± 45 [10]			
Plasma renin conc. ($U \cdot l^{-1}$)	8.4 [11]		8.8 [11]			
Plasma renin activity ($pmol \cdot ml^{-1} \cdot hr^{-1}$)				27 ± 8 [12]	45 ± 5* [12]	
				13 ± 2 [12]		15 ± 2 [12]
Dopamine beta hydroxylase ($micromol \cdot min^{-1} \cdot l^{-1}$)	25.9 ± 1.9 [13]	29.6 ± 2.5 [13]	25.1 ± 1.9 [13]			
($Units \cdot ml^{-1}$)				7.84 ± 0.24 [14]	13.4 ± 0.6*[14]	
($nmol \cdot min^{-1} \cdot l^{-1}$)				0.19 ± 0.04 [15]		0.19 ± 0.03 [15]
$^{C}Catecholes/^{C}Creatinine$	1.06 ± 0.1 [16]	1.81 ± 0.2* [16]	1.01 ± 0.2 [16]			
Plasma norepinephrine ($ng \cdot ml^{-1}$)	424 ± 193 [17]	411 ± 193 [17]		1.8 ± 0.14 [14]	2.93 ± 0.35*[14]	

1 Frohlich, Tarazi and Dustan 1969.

2 Trippodo, Walsh and Frohlich 1978.

3 Frohlich and Pfeffer 1975.

4 Manger, Werner, Freedman et al. 1974.

5 Azar, Johnson, Bruno et al. 1977.

6 Hansen 1968.

7 Kannel, Brand, Skinner et al. 1967.

8 Tobia, Walsh, Tadepalli et al. 1974.

9 Nishiyama, Nishiyama and Frohlich 1976.

10 Safar, Chau, Weiss et al. 1976.

11 Padfield, Brown, Lever et al. 1975.

12 Vincent, Dupont and Sassard 1976.

13 Aoki, Tazumi, Yoshida et al. 1975.

14 Oparil, Erinoff, Cutilletta 1976.

15 Nagatsu, Kato, Numatsa et al. 1974.

16 Kuchel, Cuche, Buu et al.1976

17 Sever,Birch,Osikowska et al. 1977.

18 Tarazi,Dustan,Frohlich et al. 1970.

PROPOSED MECHANISMS FOR SPONTANEOUS HYPERTENSION[1]

Changes in the Vasculature

Since hypertension will have a direct effect on arterial vascular wall stress, one may presume a priori that with hypertension there may be structural changes in the vasculature. Increased total peripheral resistance, calculated as the quotient of the pressure drop across the vascular system and the cardiac output, has been consistently observed in both humans with established hypertension (Frohlich, Tarazi and Dustan, 1969) and in the SHR rat (Frohlich and Pfeffer, 1975). An increase in total peripheral resistance may be due to one of several factors or to a combination of several factors: an increase in blood viscosity, a decrease in vascular diameter, a decrease in vessel number or an increase in vessel length.

The SHR rat has an increase in red blood cell count (Manger, Werner, Freedman et al., 1974) and hematocrit (Azar, Johnson and Bruno, 1977). Further, workers at the Cleveland Clinic (Sen, Hoffman, Stowe et al., 1972) have shown a highly significant linear relation between red blood cell count and systolic blood pressure in the SHR rat. Still, the degree of erythrocytosis seen in the SHR rat is minor. If I use data for human blood (Wells and Merrill, 1961) to calculate the effect of the observed erythrocytosis on viscosity, I calculate that there should be only a 6% increase in viscosity. Total peripheral resistance in the SHR rat is observed to be at least 50% greater than in the WKY. In addition, I shall describe work by Folkow (Folkow, Hallback, Lundgren et al., 1970a) which

[1] In this report, I shall use "spontaneous hypertension" to mean hypertension in the SHR rat.

shows that vascular resistance is elevated in the SHR rat even during perfusion with an artificial medium. Until more work is done defining blood viscosity in the SHR rat, it seems best to use altered vascular resistance to account for most of the elevation in total peripheral resistance in the SHR rat.

In addition to an increased total peripheral resistance in the SHR rat, specific vascular beds with greater resistance in the SHR rat than in the WKY rat include the coronary vessels (Noresson, Hallback and Hjalmarsson 1977), stomach, small intestine, liver, adrenals and lung (Tobia, Walsh, Tadepalli et al., 1974) and the kidney (Tobia, et al., 1974; Gothberg, Hallback, Lundin et al., 1976). An important question is whether this increased vascular resistance precedes or succeeds hypertension in the SHR rat.

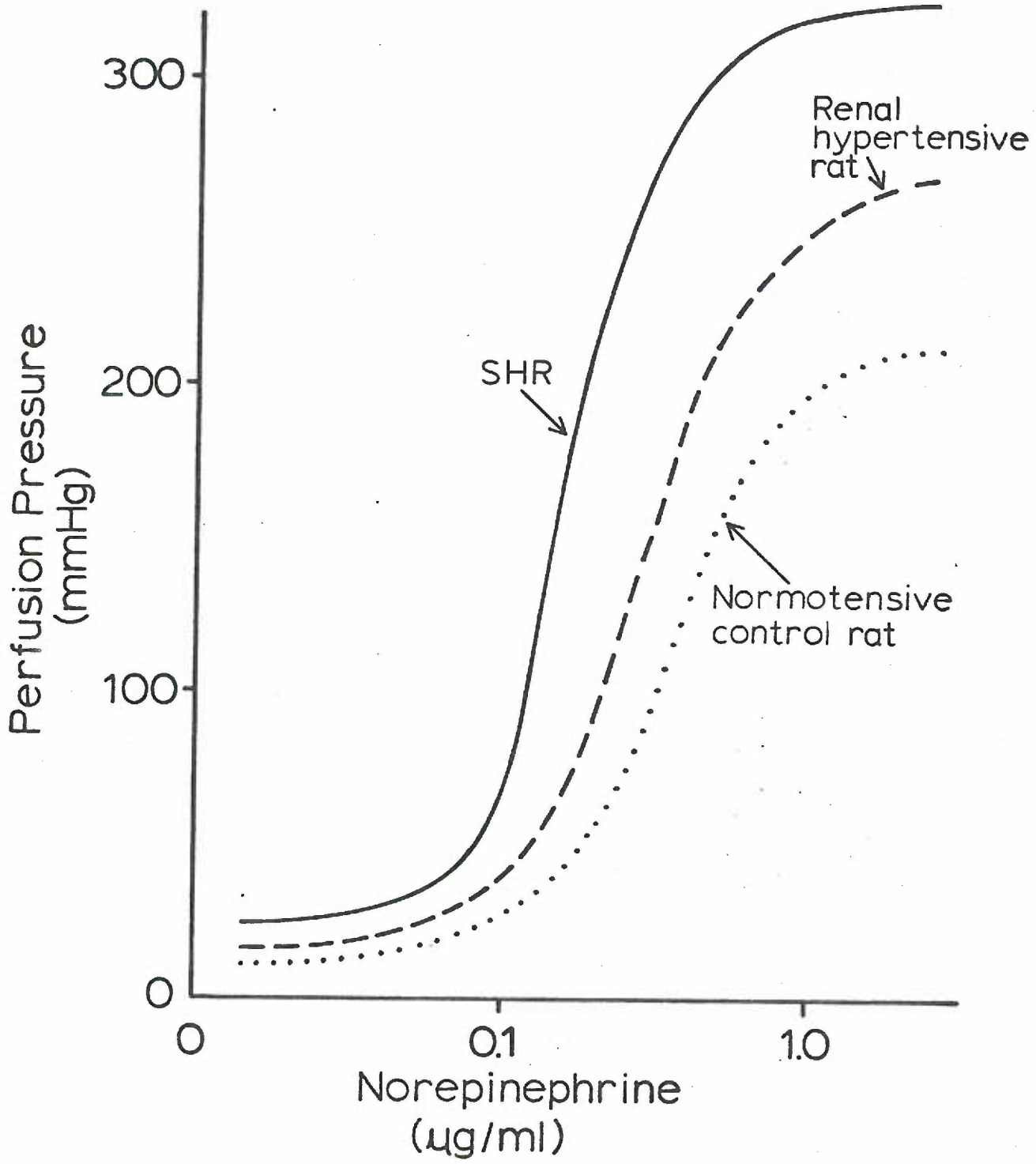
Folkow (1975) believes that increased total peripheral resistance is secondary to and adaptive for hypertension. The functional stress placed on the vasculature by hypertension induces hypertrophy of the media of resistance vessels in a positive-feedback manner. The medial hypertrophy is qualitatively much different than the vascular lesions seen in malignant hypertension (Brunner and Gavras, 1975) which may be influenced by humoral factors in addition to blood pressure. Morphologic studies of vascular architecture are plagued with questions concerning the changes attending vessel isolation and post-fixation artifacts. While some workers have found an increased wall thickness and decreased lumen diameter (Short, 1966) and a decreased number of small arterioles (Hutchins and Darnell, 1974) in the SHR rat, there is a recent report that there is no difference in vessel wall thickness between SHR rats

and WKY rats (Bohlen and Lobach, 1978).

If the only change in the vascular system is an increase in medial thickness and the mass of the contractile elements, Folkow predicts these functional consequences: 1) there will be a normal threshold or sensitivity to exogenous vasoconstrictor agents such as norepinephrine, since he assumes that the number of receptors and their avidity for vasoactive agents is not altered in hypertension, 2) the resistance at both maximal dilation and maximal constriction will be increased if medial hypertrophy encroaches on the lumen and 3) a given change in the dose of a vasoconstrictor agent will produce a greater than normal change in resistance since the increased mass of vascular smooth muscle will contract with greater force (Folkow, Grimby and Thulesius, 1958; Folkow, Hallback, Lundgren et al., 1970b). Figure 6 shows that the predictions of the structural model are confirmed in hind-limb perfusion studies of two-kidney Goldblatt rats (Lundgren, Hallback, Weiss et al., 1974) and in SHR rats (Folkow, Hallback, Lundgren et al., 1970a). The duration and severity of hypertension are not equivalent in the two models and an artificial perfusion fluid was used but the effects of norepinephrine on vasculature resistance are clearly different when the limb is from a hypertensive animal.

Controversy abounds concerning vascular reactivity in hypertension (Bohr, 1974). Whereas most workers do find an increased peripheral resistance in vivo, it is not known whether the vascular changes in SHR rats are secondary to hypertension or contribute to the initiation of hypertension. It is also not known whether the response of hypertensive vascular smooth muscle to vasoconstrictor agents is different from the

Figure 6: Mean perfusion pressure in rat hind-limbs which were perfused with various concentrations of norepinephrine in Tyrode solution at a constant flow rate of 10 $ml \cdot min^{-1} \cdot 100g^{-1}$. The solid line is from SHR rats (Folkow, Hallback, Lundgren et al., 1970a). The dashed line is from renal hypertensive rats (Lundgren, Hallback, Weiss et al., 1974). The dotted line is from normotensive Wistar control rats (not WKY rats).


300
200
100
0
Perfusion Pressure
(mmHg)
Renal
hypertensive
rat
SHR
Normotensive
control rat
0
0.1
1.0
Norepinephrine
(μg/ml)

response of normotensive vascular smooth muscle. Some workers find an increased sensitivity to vasoconstrictor agents in isolated SHR rat vessels and in perfused hind-limbs, depending on the specific vasoconstrictor or vasodilator used (Lais, Shaffer and Brody, 1974; Lais and Brody, 1975). Others (Spector, Fleisch, Maling et al., 1969; Shibata, Kurahashi and Kuchii, 1973; Hansen and Bohr, 1975) find a reduced response to vasoconstrictor agents in perfused SHR rat vessels. Folkow reports that there is no difference in vascular sensitivity to vasoconstrictors between SHR rats and WKY rats (Folkow, Hallback, Lundgren et al., 1970a) and asserts that the increased response to vasopressor agents exhibited by the SHR rat is due to increased vascular smooth muscle mass.

In agreement with his thesis that the changes in vascular resistance are secondary to and accentuated by hypertension, Folkow was able to prevent the accentuated pressor response to norepinephrine that is usually seen with hypertension by artificially producing regional hypotension. The aortas of 3 week old SHR rats and normotensive Wistar control rats (NCR) were ligated distal to the renal arteries; producing hypotension in the hind-limbs. These limbs were isolated and perfused at 9 and 19 weeks with various concentrations of norepinephrine in an artificial medium (Folkow, Gurevich, Hallback et al., 1971). Both NCR and SHR limbs showed similar and subnormal resistance at all concentrations of norepinephrine.

Bohr did not confirm Folkow's hypothesis in isolated vascular strips (Hansen and Bohr 1975). He ligated one external iliac in SHR rats, producing hypotension in only one leg and then studied the reactivity of vascular strips perfused with varying concentration of calcium. He

found that vascular smooth muscle from both femoral arteries responded in a similar fashion. It should be remembered that he was not specifically examining the resistance vessels. In isolated perfused portal veins (protected from hypertension) from 5 month old SHR rats and Wistar rats, Greenburg and Bohr (1975) found an increased rate and strength of spontaneous contraction with SHR veins. They also found a normal ED_{50} but an enhanced maximal tension to several vasoconstrictors including epinephrine, norepinephrine, Ba and Sr. The SHR vascular smooth muscle had a lowered threshold to PGA_2- and PGE_2-induced vasoconstriction. These inconsistent data in vascular smooth muscle from a protected region of the circulation suggest some specific changes in vascular smooth muscle but it is not clear whether these alterations were present before hypertension or are secondary manifestations of hypertension and therefore do not contribute to the initiation of hypertension. A report of altered in vitro reactivity of SHR stomach fundus smooth muscle (Altman, DuPonte and Worcel, 1976) suggests that smooth muscle alterations are not secondary to hypertension.

In the two-kidney Goldblatt rat, Folkow (Lundgren, Hallback, Weiss et al., 1974) examined the cause-effect relation of hypertension and vascular changes. He measured the temporal changes in isolated hindlimb resistance and ventricular mass after renal artery clipping. Significant changes in blood pressure preceded the vascular and cardiac changes by several weeks.

Folkow's whole-limb studies focus on a different type of vessel than do the studies which look at isolated conduit vessels. Until more work is done on the isolated characteristics of the resistance vessels,

it will not be possible to resolve the apparent conflicts between these two lines of research. Perhaps all that can be said at this time is that the vasculature of the mature SHR rat is hyper-responsive to certain exogenous vasoactive substances. Medial hypertrophy is one possible explanation for the hyper-responsiveness.

When assessing the importance of these structural and functional differences in the vasculature of hypertensive animals we must remember a conclusion from Guyton's analysis (Guyton and Coleman, 1969):

> "... changing the arterial resistance (without changing renal arterial resistance) theoretically will have no effect whatsoever on the final equilibrium level of arterial pressure."

Before specifically considering the status of the kidney in the SHR rat, I will review several other proposed mechanisms for hypertension in the SHR rat. In each case it must be recognized that any given neural-hormonal factor will be effective in the pathogenesis of chronic hypertension only to the extent that the relationship between arterial pressure and renal sodium and water excretion rate is changed.

Baroreceptors

Baroreceptors are important in the short-term regulation of mean arterial pressure. Responses mediated by the baroreceptors will minimize the transient changes in pressure that will occur with, for example, acute stress and postural changes.

It is not likely, however, that baroreceptor function is responsible for either the initiation or the maintenance of hypertension in the SHR rat. This is so because baroreceptors normally adapt to any change in pressure. (Cowley, Liard and Guyton, 1973). Within 48 hours after aortic coarctation in the rat, the baroreceptors will have reset to the elevated supracoarct pressure (Krieger, 1970). The threshold pressure is the lowest pressure which will induce aortic nerve activity. With coarctation, the threshold pressure is raised so that the relation of threshold pressure to prevailing pressure is the same in the hypertensive state as it had been before coarctation. The reverse phenomenon, the resetting of the threshold to a lower pressure after hypertension is reversed, is even more rapid, occurring within six hours (Salgado and Krieger, 1976).

Nosaka and Okamoto (1970) observed an apparent resetting of the baroreceptors in the SHR rat. While the maximal firing rate of the aortic depressor nerve was the same in 8 to 10 month old SHR rats and WKY rats, the perfusion pressure associated with any given firing rate was higher in the SHR rats than in the WKY rats.

There is, then, little evidence that the baroreceptors play a significant role in the development or maintenance of hypertension in the SHR rats. In fact, sinoaortic denervation produced the same increase in arterial pressure in both SHR rats and WKY rats (Okamoto, 1969), indicating that baroreceptor activity may be the same in SHR and WKY rats.

Response to Environmental Stress

Our knowledge of behavior and response to stress as factors in the pathogenesis of hypertension in humans and the SHR rat is very limited. Although few studies have been carried out on the behavior of the SHR rat, all indicate that the SHR rat is more aggressive and also more sensitive to environmental stimuli than are WKY rats.

Shimamoto and Nagaoka (1972) observed that SHR rats rear up on their hind legs more frequently than do WKY rats; this may correlate with increased cortical activity. They also found that SHR rats were more aggressive than WKY rats. SHR rats demonstrated increased mouse-killing behavior when a mouse was introduced into the cage with a rat which had been isolated. Campbell and DeCara (1977) also observed behavioral differences between SHR rats and WKY rats when they paired a tone (conditioned stimulus or CS) with a tail shock (unconditioned stimulus or US). They found that 28 week old SHR rats and those WKY rats with the highest systolic blood pressures showed much more motor avoidance behavior to the CS than did the WKY rats with the lowest blood pressures. Increased motor activity as such does not provide a complete explanation for spontaneous hypertension since neither 11 to 12 week nor 16 to 18 week old SHR rats showed more pressor response to treadmill exercise than that which was seen with WKY rats (Baum and Shropshire, 1975). Eichelman, Dejong and Williams (1973) confirmed the increased aggressiveness in 9 to 13 week old SHR rats as measured by shock-induced fighting behavior. Additionally, SHR rats would jump in response to a smaller electric shock than the shock required to make WKY rats jump. Significantly, neither the Dahl salt-sensitive nor a renal hyper-

tensive rat showed increased aggression or sensitivity to pain when compared to their appropriate controls.

Other evidence of an enhanced response to environmental stimuli in the SHR rat are the observations that SHR rats raised in isolation (Hallback, 1975a) or in dark rooms (Lais, Bhatnagar and Brody, 1974) have a lower blood pressure than that of SHR rats raised in group cages or 12 hour light-dark cycles; this effect is not seen in normotensive rats. In SHR rats which were subjected to continuous noise every night after the age of 13 to 17 weeks, Borg and Moller (1978) found no increase in blood pressure when compared with SHR rats kept in quiet cages. Although the noise produced a significant hearing loss, habituation to the noise was apparent so that noise was not an alerting stimulus under these conditions. Thus, noise per se is probably not hypertensinogenic but it may provoke hypertension if it is an alerting stimulus.

An important finding was that the SHR rat was more likely than the normotensive rat to show a pressor response to acute stresses such as flashing lights and loud noises (Hallback and Folkow, 1974). An enhanced pressor response to acute stress was not seen in two other rat models of hypertension: the two-kidney Goldblatt rat (Hallback and Folkow, 1974) and the Milan hypertensive rat (Hallback, Jones, Bianchi et al., 1977). The acute pressor response to stress in the SHR rat was more pronounced in 10 to 11 week old rats with developing hypertension than in older rats. Thus, Hallback and Folkow (1974) hypothesized that a genetic predisposition of young SHR rats to have an enhanced pressor response to alerting stimuli may be a crucial factor in the etiology of spontaneous hypertension.

Autonomic Nervous System

Several lines of evidence indicate that the autonomic nervous system is a central factor in the development of hypertension in the SHR rat. Specifically, there appears to be enhanced activity of the sympathetic nervous system in young SHR rats with developing hypertension.

One measure of the activity of the sympathetic nervous system in the SHR rat is the plasma and tissue concentrations of active catechols or their associated enzymes. Phenylethanolamine N-methyl transferase (PNMT) is an enzyme involved in the conversion of norepinephrine to epinephrine. PNMT content in the nuclei tractus solitarii and norepinephrine content of superior cervical ganglia were higher in the SHR rats than in WKY rats after 9 weeks but not at 2 or 4 weeks of age (Gianutsos and Moore, 1978). Other measures of sympathoadrenal activity such as plasma dopamine beta hydroxylase and norepinephrine levels (Grobecker, Roizen, Weise et al., 1975) were elevated in young SHR rats at about the age that hypertension was developing.

Direct recording of post-ganglionic nerve activity of a cervical sympathetic nerve, the greater and least splanchnic nerve, the renal nerve and the splenic nerve showed elevated activity in each nerve in the SHR rats at 16, 24 and 40 weeks when compared with age-matched WKY rats (Judy, Watanabe, Henry et al., 1976). The nerve activity increase is parallel with the hypertension. When Oparil and her colleagues (Cutilletta, Erinoff, Heller et al., 1977) injected rats on the first and seventh day after birth with nerve growth factor antiserum (NGFAS), she found that the development of hypertension was prevented for at least 80 days in the SHR rat. Blood pressure of WKY rats with NGFAS

was not different than that of saline-injected rats.

In contrast to these studies, which support a pathogenic role for the sympathetic nervous system in spontaneous hypertension are a number of studies in SHR rats older than 17 weeks which indicate that the sympathetic nervous system is not required for the maintenance of hypertension. It is important to keep the age of the rat in mind if we are to separate cause from consequence. Iriuchijima (1976) found that 23 week old SHR rats remained hypertensive several hours after spinal cord section at C7 to T1. Lumbar sympathectomy produced only a modest fall in total peripheral resistance in 6 to 9 month old SHR rats (Lais, Schagger and Brody, 1974).

By a more drastic procedure, however, Mizogami, Suzuki and Sokabe (1972) were able to demonstrate a role for the nervous system in maintaining the difference in blood pressure between the SHR rats and WKY rats. Blood pressure was identical in 17 to 20 week old SHR rats and WKY rats after combined pithing, decerebration and vagotomy.

Bunag, Eferakeya and Langdon (1975) found that the conscious 18 to 20 week old SHR rats showed a greater pressor response than did normotensive Wistar rats to stimulation of the posterior hypothalamus. This may mean that the SHR rat is also hyper-responsive to other stimuli that can activate the sympathetic nervous system.

On the whole, the work in this area seems to indicate that the sympathetic nervous system is hyperactive in the young SHR rat and that it may play a role in the development of spontaneous hypertension. Most observations are consistent with Yamori's (1976) suggestion that "... neurogenic mechanisms are important for initiation, whereas the non-neurogenic mechanisms are involved in the maintenance of spontaneous hypertension."

Prostaglandins and Natriuretic Hormone

Muirhead (1975) has noted that, although renal disease may promote hypertension, there appears to be an antihypertensive function of the kidney that is hormonally-mediated. Grollman, Muirhead and Vanatta (1949) showed that, whereas bilateral nephrectomy produced sustained hypertension in dogs maintained with an artificial kidney, non-nephrectomized dogs with ureteral ligation or ureteral-caval anastomoses did not develop sustained hypertension. The nature of the renal hormone that is postulated to mediate this antihypertensive effect is not established. It has been proposed that the antihypertensive hormone is a member of the family of prostaglandins (PG) which are known to be synthesized from arachidonic acid in the renal medulla.

Although PGE_2 is a vasodilator in most vascular beds, it is a vasoconstrictor in the rat kidney (Malik, 1978). Nonetheless, infusion of large amounts of PGE_1, PGE_2 or arachidonic acid into the SHR rat acutely lowered blood pressure to a value that was not different from the blood pressure in WKY rats (Cohen, Sztokalo and Hinsch, 1973). This might be due to a direct effect of the prostaglandins to lower peripheral vascular resistance.

There are also reports that certain of the prostaglandins may promote sodium retention. This may mean that the effect of prostaglandins on the kidney is to promote rather than to prevent hypertension. Inhibition of prostaglandin synthesis increased sodium concentration in the rat renal medulla (Ganguli, Tobian, Azar et al., 1977). PGE_2 inhibited sodium transport in isolated rabbit collecting tubules (Stokes and Kokko, 1977).

The classic factors in the regulation of renal sodium excretion include: 1) aldosterone, 2) glomerular filtration rate and 3) transtubular pressure gradients (Bricker, Schmidt, Favre et al., 1975). The natriuretic response to volume expansion cannot be explained entirely by these factors (deWardener, 1977). The presence of a natriuretic hormone responsible for this natriuresis has been repeatedly demonstrated by cross-perfusion studies and by assay in an isolated kidney of plasma or urine from volume-expanded animals. Natriuretic hormone is not a prostaglandin and may be secreted by the brain but its chemical nature and site of secretion have not been established.

Neither prostaglandins nor natriuretic hormone have yet taken a prominent place in the list of factors which are candidates as agents in the pathogenesis of hypertension. Research on these hormones, however, is deservedly extensive and we may find that they are critical to our understanding of the mechanisms of hypertension both in SHR rats and in humans.

The Renin-angiotensin System

Early reports recognized the ability of a substance of renal origin to produce acute elevation of blood pressure and suggested that this substance (renin) may play a role in renal vascular hypertension (Goldblatt, 1937). Much of what has subsequently been learned about renin and hypertension has failed to extend our understanding of the pathogenetic role of renin in human essential hypertension. Laragh and his colleagues (Brunner, Laragh, Baer et al., 1972) acknowledged the heterogeneity of human essential hypertension with their classification of patients as having a low, normal or high plasma renin activity (PRA), relative to sodium excretion rate.

The SHR rat is genetically rather homogeneous and the results of studies of PRA have been more uniform in the SHR rats than in human populations. Freeman, Davis, Varsano-Aharon et al. (1975) reported that PRA in 7 to 8 week old pentobarbital-anesthetized SHR rats was significantly less than in WKY rats. Shino and Sokabe (1976) found relatively lower PRA in the conscious SHR rat at all ages from 5 to 30 weeks. In a recent study from Okamoto's lab (Matsunaga, Komuro, Yamamoto et al., 1977), PRA in conscious unrestrained rats was the same in SHR rats and WKY rats from 7 to 43 weeks.

The only reports of enhanced activity of the renin-angiotensin system in the SHR rat come from work with young rats. Sinaiko and Mirikin (1974) and Sen, Smeby and Bumpus (1972) find that, in rats younger than 3 weeks and younger than 7 to 8 weeks, respectively, the SHR kidney had a higher renin content than that of the WKY kidney. One criticism of these studies is that they used anesthetized animals. Fur-

ther, it is not valid to conclude that when kidney renin content is high, peripheral and renal activation of the renin-angiotensin system will occur in the young SHR rats. There may even be a negative correlation between PRA and kidney renin content (Boucher, Rojo-Ortega and Genest, 1977).

The consistency of results in studies of PRA in the SHR rat should not lead us to think that we understand the role of the renin-angiotensin-aldosterone system in spontaneous hypertension. PRA is only one, and perhaps a misleading, indicator of the activity of this system (Boucher, Rojo-Ortega and Genest, 1977). Local concentrations, presence of inhibitors and the site of action of hormonal elements of this system remain to be described before we can assign a role in the development or maintenance of hypertension to this system.

Reports of normal plasma aldosterone levels are consistent with the impression gained from PRA studies which indicate that the renin-angiotensin-aldosterone system is not solely responsible for hypertension in the SHR rat. Freeman, Davis, Varsano-Aharon et al. (1975) and Moll, Dale and Melby (1975) found that plasma aldosterone concentration in the hypertensive SHR rat was the same as or less than the concentration in normotensive rats.

The finding that PRA was elevated in young SHR rats will need to be confirmed in conscious rats and the relation between PRA and fluid volume control must be clarified before we will know whether alterations in the renin-angiotensin system are important in the pathogenesis of spontaneous hypertension.

THE SHR KIDNEY

It should be pointed out that, although Guyton's analysis demands that hypertension must be associated with a rightward shift of the renal function curve, the mechanism by which this rightward shift occurs is not critical. The shift could be on the basis of an abnormality intrinsic to the kidney such as vascular abnormalities, lack of renal production of a vasodilator substance, excessive renal production of vasocontrictor substances or enhanced renal sensitivity to vasoconstrictor substances. On the other hand, the rightward shift of the renal function curve could occur with an otherwise normal kidney that is subjected to abnormal influences originating from extrarenal sites such as elevated sympathoadrenal function. It is not known which of these processes is at work in humans with essential hypertension or if the rightward shift of the renal function curve is due to both intrinsic renal abnormalities and abnormal renal influences which arise from extrarenal sources.

Transplantation Studies

One approach to the question of whether or not intrinsic renal abnormalities cause hypertension has been to follow the course of blood pressure after renal transplantation from hypertensive to normotensive rats or from normotensive to hypertensive rats. The results of three studies are summarized in Table 4. Because of problems with transplant rejection, Manger's group have been unable to reproduce the findings of their initial report in the SHR rat which indicated that the kidneys from older SHR rats were not hypertensinogenic (Coburn, Manger, Dufton et al., 1972).

Using hypertensive rats derived from a Wistar colony in Milan, Bianchi (Bianchi, Fox, DiFrancesco et al., 1973) showed that if a kidney from a normotensive donor rat was transplanted into a hypertensive recipient, there was a significant fall in the blood pressure of the genetically-hypertensive rat toward but not reaching normal levels. When kidneys from hypertensive donors were transplanted into normotensive recipients, there was no change in blood pressure. Kidneys from both of Bianchi's strains are histologically normal at 13 weeks of age.

Using the salt-sensitive rat derived from a Sprague-Dawley colony, Dahl, Heine and Thompson (1974) have produced strong evidence that the kidney itself is a primary contributor to hypertension. In cross-transplantation studies between salt-sensitive and salt-resistant animals, they found that it was invariably the origin of the kidney that determined the final blood pressure in the recipient. This was true both in pre-hypertensive rats (Dahl, Heine and Thompson, 1974) and in rats with hypertension of several weeks duration (Dahl and Heine, 1975). Further, when kidneys from resistant or sensitive donors were transplanted into rats with one-kidney Goldblatt hypertension, the resistant kidneys had a greater antihypertensive effect than did the kidneys from salt-sensitive donors (Tobian, Coffee, McCrea and Dahl, 1969).

Table 4: Summary of the results of renal transplantation studies in several rat models of hypertension. The host is the recipient of the transplant.

		Coburn, Manger, et al., 1972.	Bianchi, Fox, et al., 1973.	Dahl, Heine, et al., 1974.
		16-20 weeks	13 weeks	6-8 weeks
Host	Donor	SHR	Milan-Wistar	Salt-sensitive
Hypertensive	Normotensive	Hypertensive	Normal	Normal
Hypertensive	Hypertensive	Hypertensive	Hypertensive	Hypertensive
Normotensive	Normotensive	Normal	Normal	Normal
Normotensive	Hypertensive	Normal	Normal	Hypertensive

Sodium Excretion

An exaggerated natriuretic response to an oral or intravenous saline load in the absence of a significant difference in glomerular filtration rate is a consistent observation in humans with essential hypertension (Lowenstein, Beranbaum, Chasis et al., 1970; Luft, Grim, Willis et al., 1976). Studies of saline-induced natriuresis in the SHR rat lack the consistency of human studies.

Table 5 presents the evidence for exaggerated natriuresis in the SHR rat. Exaggerated natriuresis has been described in anesthetized (Dibona and Sawin, 1976; Mullins and Banks, 1976) and conscious (Willis, McCallum and Higgins, 1976) SHR rats. Others have failed to find an exaggerated natriuresis in anesthetized (Farman and Bonvalet, 1975) and conscious (Beierwaltes and Arendshorst, 1978) SHR rats. All the experiments can be criticized. In as much as Beierwaltes and Arendshorst (1978) used the most physiologic preparation, I believe that their results may be the most relevant and that the SHR rat does not respond to a saline load with an exaggerated natriuresis.

Another approach to the examination of the role of the kidney in spontaneous hypertension is to compare the renal function curve of SHR rats with that of WKY rats. Figure 7 summarizes the results from three of these studies. I have normalized all the reported renal sodium excretion rates to body weight. Each of the studies used male rats.

Table 5: Reports of exaggerated natriuresis in rats.

Authors	Control Rat	Age	Anesth.	Saline	Remarks	Critique
Farman and Bonvalet 1975	Wistar	9 wk 18-30 wk	Nembutal 50 mg/kg	2.5% BW i.v.	SHR rat has less enhanced natriuresis. All rats showed increased $U_{Na}V$ and decreased U_KV with saline infusion. Young SHR rats have fewer glomeruli per kidney than WKY rats.	Wistar control Anesthetized
Dibona and Sawin 1976 (Abstract)	WKY	14-16 wk	?	? i.v.	No change in P_{PT}, SNGFR or FF. Points to greater Na reabsorption in the loop of Henle.	Abstract Isolated kidney?
Mullins and Banks 1976	Two types of SHR; Wistar and WKY female	6-17 wks	Inactin 100mg/kg	3.5% BW i.v.	Only one type of SHR rat showed enhanced natriuresis but it was a rat with decreased GFR when young (sick?). No pattern of change in GFR with saline infusion.	Used t test inappropriately for multiple comparisons. Anesthetized
Willis, McCallum and Higgins 1976	WKY	10-12 wks	Ether to induce voiding	6 ml by stomach tube	Conscious rat with chronic caths. SHR rat showed enhanced natriuresis but no kaluresis as did the WKY rat and also humans with essential hypertension. Increased creatinine excretion rate the same in SHR and WKY rats. Points to altered aldosterone response in the SHR rat.	Imperfect urine collection method:stimulate renal nerve selectively in the SHR?
Beierwaltes and Arendshorst 1978	WKY male	13 wks	Ether then 90' recover and restraint	3% BW i.v.	SHR rat has the same RPF, increased GFR and FF vs. the WKY rat at rest. Same increase in GFR (10%) and RPF (30%) as the WKY rat. No enhanced natriuresis in the SHR rat.	Only 90' after surgery.

Tobian, Johnson, Lange et al. (1975) worked with isolated kidneys from 17 week old SHR rats and WKY rats which were perfused with blood directly from two other rats. The perfusing rats were adrenalectomized and nephrectomized. Ethyl-malonyl-thiourea was used as an anesthetic and adrenal steroids were replaced with a constant infusion of cortisol and aldosterone. Renal resistance was greater at all perfusion pressures in the SHR kidney, but sodium excretion rate was not different between SHR and WKY kidneys. Also, renal renin release from the SHR kidney was lower than from the WKY kidney. Tobian recognized that the renal function curve for the intact SHR rat must be to the right of the WKY curve. He proposed that neural influences may cause the renal function curve of the SHR kidney to be shifted to the right of the WKY kidney in the intact animal.[1]

Two more recent studies provide support for the hypothesis that the renal function curve of the SHR rat is to the right of the WKY curve in the conscious intact rat. Additionally, both studies show that the renal function curve of the intact kidney is steeper than the curve Tobian obtained from the isolated kidney and are consistent with the prediction that the SHR and WKY renal function curves are parallel.

Guyton's group (Norman, Enobakhare, DeClue et al., 1978) used 4 to 9 month old rats which were given a sodium-deficient diet and deprived

[1] I acknowledge my debt to Tobian. I have pursued some of the questions raised by Tobian, using another experimental model to examine the possible involvement of the autonomic nervous system in the sodium excretory function of the kidney.

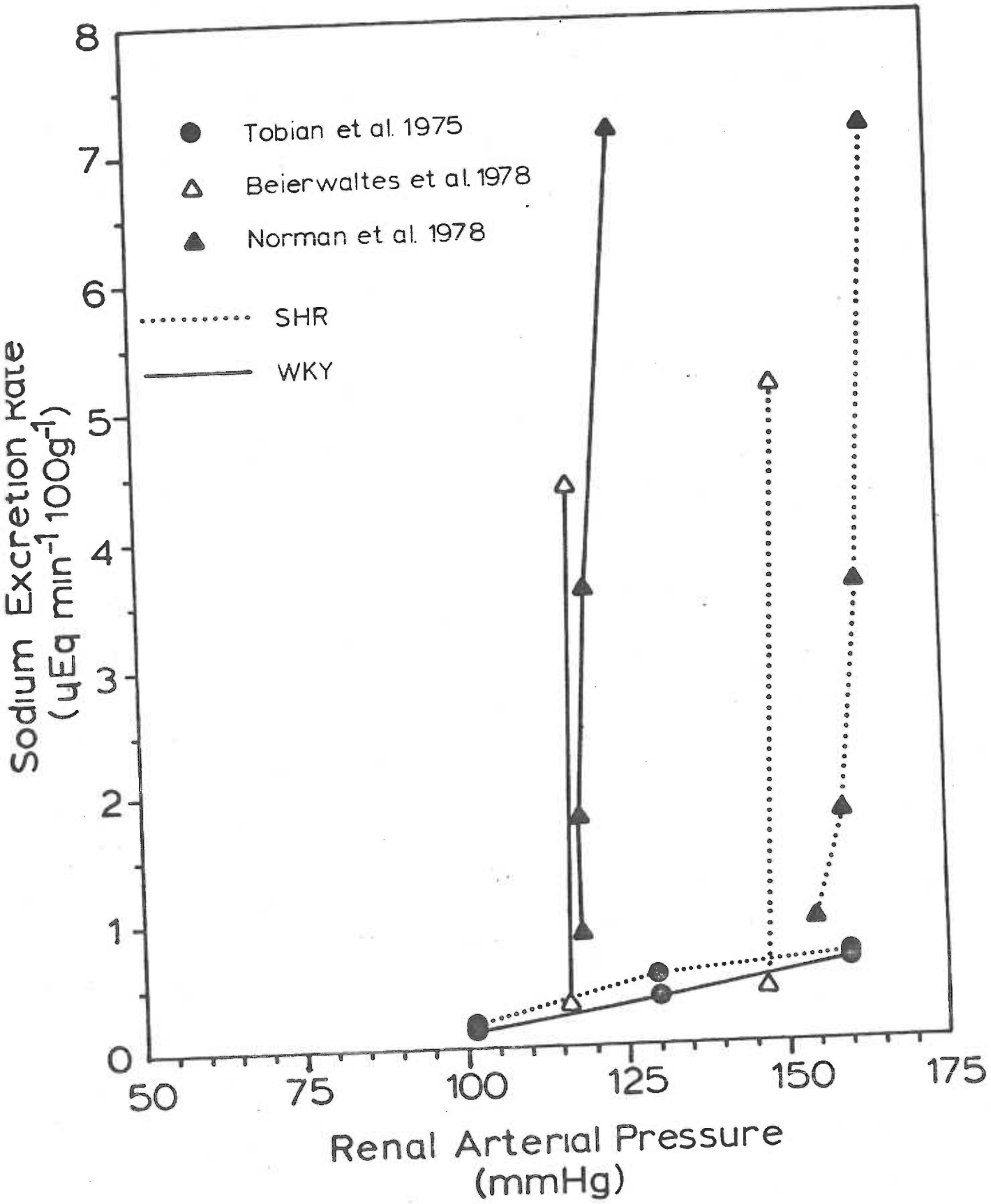
of drinking water. All water and salt intake was controlled by a continous venous infusion of saline. Mean arterial blood pressure was monitored by an indwelling femoral artery catheter. Saline infusion rate was maintained at each of several values for 24 to 48 hours; this was much longer than the 6 hours required to achieve sodium and water balance in this preparation. The renal function curve was delineated both with step-wise increases and decreases in saline infusion rate. There was no hysteresis. These workers interpret the finding that the SHR curve was parallel and to the right of the WKY curve to mean that, in the SHR rat, there is a general renal vasoconstriction since a loss of renal mass, administration of exogenous angiotensin II or of exogenous aldosterone have all been shown to shift the renal function curve to the right but also to make it less steep (DeClue, Coleman, Cowley et al., 1976).

Guyton suggests that there is considerable evidence from other studies that increased activity of the sympathetic nervous system in the SHR rat may be responsible for the rightward position of the renal function curve. We cannot, however, rule out a role for genetically-determined vascular structural changes or vascular hypertrophy secondary to hypertension which may increase renal vascular resistance and thereby place the SHR renal function curve to the right of the curve for WKY rats.

Beierwaltes and Arendshorst (1978) used 13 week old rats to examine the effect of acute volume expansion with 3 ml saline per 100g body weight i.v. on sodium excretion rate. During ether anesthesia, catheters were placed in the urinary bladder and the femoral artery and vein. Experiments were done 90 minutes after surgery in conscious restrained

rats. Urinve collection periods were 30 to 45 minutes in duration. Since there was an increase in arterial pressure after the saline infusion, we may construct a simple renal function curve for these rats by comparing the mean arterial pressure and sodium excretion rate before volume expansion to values after volume expansion.

Figure 7: Reports of renal function curves in SHR rats and WKY rats. Data from Beierwaltes et al. (1978) and Norman et al. (1978) were from conscious, intact rats. Tobian et al. (1975) used isolated blood-perfused kidneys.


Tobian et al. 1975
Beierwaltes et al. 1978
Norman et al. 1978
SHR
WKY
Sodium Excretion Rate
(μEq min⁻¹ 100g⁻¹)
Renal Arterial Pressure
(mmHg)
0
1
2
3
4
5
6
7
8
50
75
100
125
150
175

The Relation of the Kidney to Spontaneous Hypertension

We have seen that there is an altered relationship between sodium excretion rate and blood pressure in the SHR rat. We do not, however, know that the SHR kidney behaves abnormally due to a genetically-determined structural or functional renal defect. It is also possible that the kidney is responding to abnormal neural-humoral influences originating from other organs. In other words, a critical question is: "Is the rightward position of the renal function curve in the SHR rat due to a primary renal abnormality or is it due to a secondary extra-renal influence on renal function?"

Among several approaches to this question are examination of the renal nerve activity, the effects of renal denervation and the natriuretic behavior of the isolated kidney.

Renal Nerve Activity and the Renal Vasculature

Renal nerve activity may be important for the development of hypertension in the SHR rat. Although lumbar sympathectomy does not produce a persistent fall in blood pressure in 6 to 9 month old SHR rats (Lais, Schagger and Brody, 1974), reports of surgical renal denervation in 6 week (Liard, 1977) or 8 week old (Kline, Kelton and Mercer, 1978) SHR rats support a role for renal sympathetic nerve activity in the pathogenesis of but not necessarily in the maintenance of spontaneous hypertension. In these studies the onset of hypertension was delayed by the denervation; blood pressure of denervated rats was less than the pressure for sham-operated SHR rats up to the age of 20 weeks.

What are the mechanisms for the apparent involvement of renal sympathetic nerve activity in spontaneous hypertension? This question has two components. First, is the SHR kidney subjected to an elevated local concentration of adrenergic neurotransmitters or is the kidney hyperresponsive to normal levels of neurotransmitters? Second, how might renal sympathetic nerve activity promote the development or maintenance of hypertension?

Concerning the first question, Fink and Brody (1978), using histofluorescent methods, reported that there was no difference in the concentration of adrenergic terminals between SHR and WKY kidneys. It is not necessary, though, to propose an increased renal sensitivity to neural stimuli in SHR rats since direct recordings in anesthetized adult SHR rats have shown elevated renal nerve activity compared to WKY rats (Judy, Watanabe, Henry et al., 1976).

There are several considerations concerning the second question. One possibility is that there is a neurally-mediated increase in renal renin release. I have already mentioned that elevated peripheral renin is not consistently found in the SHR rat. Could there be an increase in that component of proximal sodium reabsorption that is dependent on direct adrenergic stimulation (DiBona, 1977)? There are no reports of studies on this aspect of sodium reabsorption in the SHR rat.

Finally, renal sympathetic nerve activity may, by raising pre- and post-glomerular vascular resistance, lower GFR and thereby promote sodium retention and hypertension. Certainly, renal vascular resistance in situ is elevated in both young and old SHR rats (Tobia, Walsh, Tadepalli et al. 1974; Nishiyama, Nishiyama and Frohlich,1976). Isolated perfused kidneys from 30 weeks old SHRs have the same resistance as normotensive rats unless norepinephrine is included in the perfusate (Folkow, Hallback, Lundgren et al., 1971). Folkow explains the elevated resistance with various vasoconstrictor agents on the basis of hypertrophy of the vascular smooth muscle.

Tobian's group (Azar, Johnson, Bruno et al., 1977) analyzed single nephron hemodynamics in 17 to 18 weeks old SHR and WKY rats. They found that both afferent and efferent arteriolar resistance was greater in the SHR rat than the WKY rat and that glomerular capillary pressure and single nephron GFR were normal. The SHR kidney does, however, have an intact tubuloglomerular feedback mechanism and will respond normally to a fall in blood pressure. Workers from Munich (Ploth, Dahlheim, Schmidmeier et al., 1978) found that 12 to 20 week old SHR rats could

maintain a constant single nephron GFR (as measured with distal tubular fluid collections) when kidney perfusion pressure was acutely lowered by partially clamping the aorta.

In view of the considerable evidence that there is a functional increase in renal vascular resistance in the SHR rat, it seems surprising that there have been no reports of structural alterations in the SHR rat's renal vasculature except in rats which were quite old (Freis and Ragan, 1975). Using Poiseuille's equation for resistance with laminar flow, we can calculate that a 10% decrease in vascular radius is sufficient to account for the observed 50% increase in total peripheral resistance of SHR rats compared to WKY rats. Such small differences in vascular radii would be difficult to detect histologically.

THE OBJECTIVES OF THIS WORK

The Problem

Having established the existence of an abnormal in vivo relation between sodium excretion rate and mean arterial pressure in the hypertensive SHR rat, I am concerned with two questions:

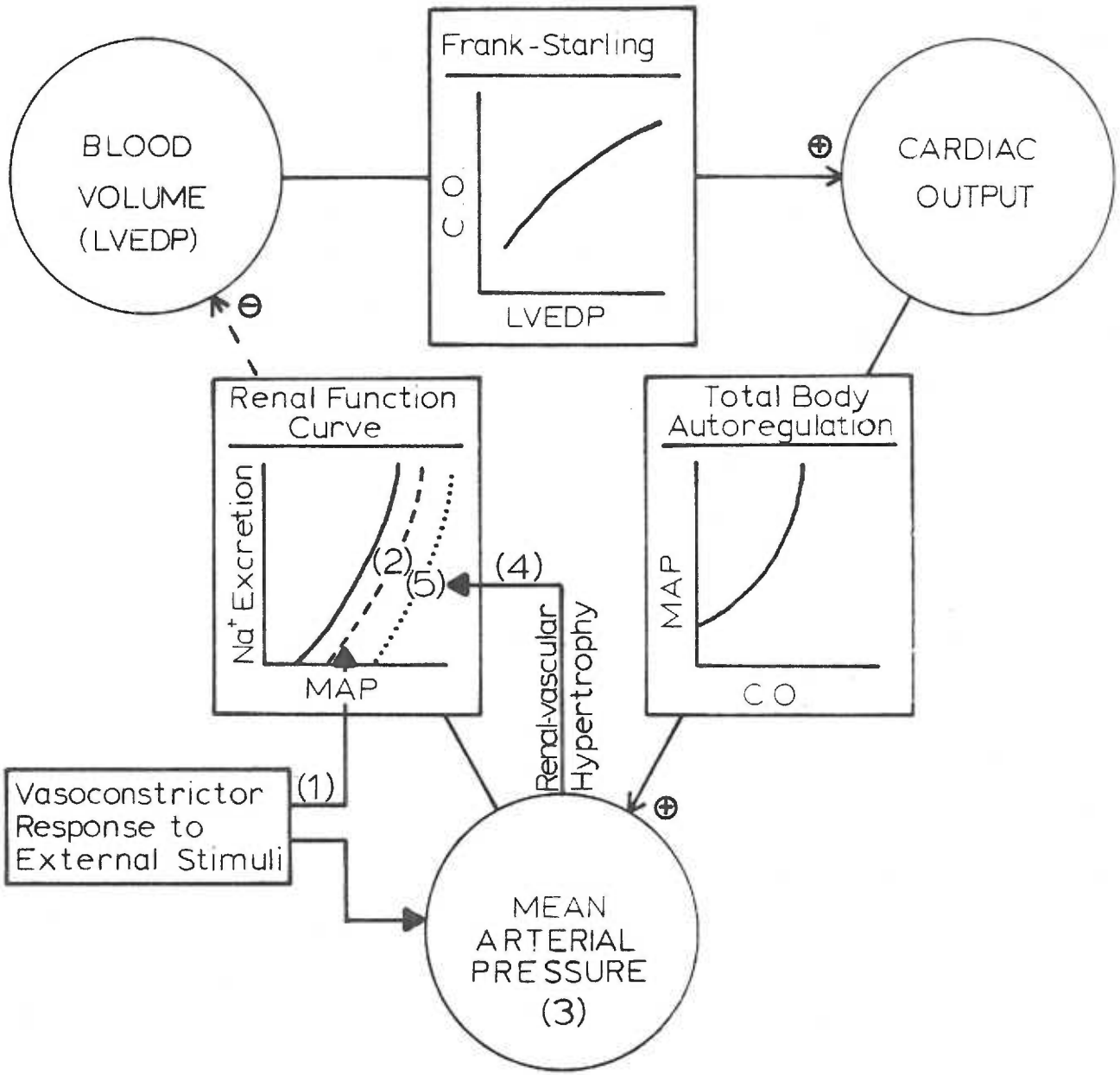
1) What is the nature of the renal function curve abnormality in the SHR rat? Is there an intrinsic renal pathology (such as hypertrophy of the renal vascular smooth muscle) or do extrarenal influences (such as the sympathetic nervous system or aldosterone) act on the kidney to change the renal function curve?
2) Is the abnormal in vivo renal function curve the cause of or a consequence of hypertension?

The Hypothesis

My hypothesis is a synthesis of existing data which are related to the problem of spontaneous hypertension. Refer to the previous section on the "Proposed Mechanisms for Spontaneous Hypertension" for more details on the data on which the hypothesis is based. Predictions made from the hypothesis are the basis for the definition of the design of these experiments. Bracketed numbers in the text refer to Figure 8 which is a statement of my hypothesis.

Hyper-responsiveness to environmental stress at an early age is the feature of the SHR rat that is a primary predisposing factor for the development of hypertension. The SHR rat shows an enhanced vascular response to alerting stimuli from the environment [1] that is expressed both as peripheral and renal vasoconstriction. This pressor response is mediated by the sympathetic nervous system. As a consequence of renal vasoconstriction in the young SHR rat, the renal function curve becomes shifted to the right [2] . A rightward shift of the renal function curve will lead to decreased sodium excretion at normal arterial pressures. Therefore, there will be sodium retention and increased blood volume. The elevated blood volume will act to raise cardiac output and, thence, blood pressure [3] . In time, the peripheral and renal arterioles respond to the stress of the elevated blood pressure with smooth muscle hypertrophy [4] . By the age of 20 weeks, when hypertension is fully developed, the rightward shift of the renal function curve [5] is no longer due to enhanced neural activity but is the result of the vascular hypertrophy which has occurred secondary to the hypertension.

Figure 8: The relation of my proposed explanation for the initiation and maintenance of hypertension in the SHR rat to the systems involved in the regulation of arterial pressure. The numbers follow the progression from the initiation of hypertension in the young SHR rat to the establishment of fixed hypertension in the mature rat. Refer to the text for a more complete description of the numbers.


BLOOD
VOLUME
(LVEDP)
Frank-Starling
C O
LVEDP
CARDIAC
OUTPUT
Renal Function
Curve
Na+ Excretion
MAP
(2)
(5)
(4)
Renal-vascular
Hypertrophy
Total Body
Autoregulation
MAP
C O
Vasoconstrictor
Response to
External Stimuli
(1)
MEAN
ARTERIAL
PRESSURE
(3)

I propose that there are two primary genetic alterations in the SHR rat. First, the central nervous system of the SHR rat is hyper-responsive to alerting environmental stimuli. Second, the vasculature of SHR rats hypertrophies more rapidly than normal in response to a pressure stress.

This hypothesis is not without precedent. Indeed, the opinion that renal damage is a consequence of hypertension is at least one hundred years old (Mahomed, 1879). Brown (Brown, Lever, Robertson et al., 1976) proposed that the vasculature will hypertrophy more rapidly in response to transient episodes of elevated arterial pressure in people with a genetic predisposition to hypertension than in normotensive people. This creates the same kind of positive feedback situation that I propose is present with the SHR rat. That is, hypertension induces renal vascular hypertrophy which, by moving the renal function curve farther to the right, acts to resist any return to a lower blood pressure.

Support for part of this hypothesis has recently come from Folkow's laboratory (Folkow, Gothberg, Lundin et al., 1977a). They perfused isolated SHR rat and Wistar control kidneys with oxygenated Tyrode's solution which contained high molecular weight dextran. Sodium nitroprusside was used as a smooth muscle relaxant and the GFR was measured with Cr-EDTA. Kidneys were perfused at various pressures from 50 to 125 mmHg and the relation between perfusion pressure and GFR (normalized to kidney weight) was plotted as a curve. The curves for SHR rat and Wistar control kidneys at 5 weeks of age, when the SHR rat has nascent hypertension, were superimposable but the curve for kidneys from 13 to

17 week old hypertensive SHR rats lay significantly to the right of and parallel to the Wistar control curve.

Folkow's data supports part of my hypothesis in that the data indicate that the isolated kidney of the young SHR rat is normal whereas the kidney from the older SHR rat may have a fixed increase in vascular resistance even when it is removed from extrarenal neural-humoral influences. The interpretation of this work is based on the positive association between renal plasma flow, glomerular filtration rate and sodium excretion rate. That is, I have inferred that an increase in renal plasma flow and sodium excretion rate will accompany an increase in glomerular filtration rate.

In other work, Folkow, Gothberg, Lundin et al. (1977b) showed that the rightward shift of the renal function curve is not a general response to hypertension. Four to five weeks after surgery to produce two-kidney Goldblatt hypertension in Wistar rats, the unclipped kidney was perfused in the same manner as that of the previously-mentioned report for SHR rats. In this case there was no difference in the positions of the renal function curves for renal hypertensive kidneys and the normotensive control kidneys. It may be that hypertension alone is not sufficient for the rightward shift of the renal function curve and that a genetically-determined increase in the rate of renal vascular hypertrophy is also required. These observations indicate that hypertension may not cause renal vascular hypertrophy in renal hypertensive rats. In contrast, Folkow (Lundgren, Hallback, Weiss et al., 1974) reported data which may mean that there is vascular hypertrophy in the hind-limbs of renal hypertensive rats.

Strategy and Predictions

This work is a study of the renal function curves in unanesthetized SHR and WKY rats at ages from 8 to 32 weeks. An important difference between my work and that which has been reported previously is that I used conscious rats. I have described many reports which indicate that central nervous system activity has some influence on the development of spontaneous hypertension. In addition, I will report that I found that pentobarbital produced significant hypotension which was independent of the effects of surgery in adult SHR and WKY rats. Therefore, anesthetics should be avoided in studies of the pathophysiology of hypertension in the SHR rat. More generally, Vatner and Braunwald (1975) have described the hazards of using anesthetics in experiments which examine cardiovascular parameters.

I used hexamethonium as a ganglionic blocker to eliminate sympathetic influences on the renal function curve since I proposed that the renal function curve for the older SHR rats would be to the right of the WKY curve even without sympathetic influences on the kidney.

By my hypothesis, I predict that, after ganglionic blockade, the renal function curve for the SHR rat will lie only slightly to the right of the WKY curve when hypertension in the SHR rat is nascent. Even without sympathetic influences on the kidney, the SHR rat's renal function curve will shift progressively to the right as hypertension persists and becomes more severe. If the rightward shift of the renal function curve in the SHR rat is a consequence of renal vascular hypertrophy, I expect that after ganglionic blockade the renal function curves for SHR rats will be shifted to the right of, and parallel to,

those of WKY rats of similar age.

These are my predictions. How might I interpret deviations from these predicted results? If, after ganglionic blockade, there is no difference between the renal function curves of SHR and WKY rats, I would conclude that, at that age, the sympathetic nervous system has a major role in the separation of the curves which is observed without ganglionic blockade. If a separation of the curves persists after ganglionic blockade, I can assume that differences in response to neural influences are not solely responsible for the separation of the SHR and WKY renal function curves in the undisturbed rat. If a separation of the renal function curves after ganglionic blockade is present in young rats with nascent hypertension, this could be taken as evidence that the kidney itself is responsible for the initiation of hypertension in the SHR rat. Finally, a difference in the slopes of the renal function curves after ganglionic blockade would call attention to a possible involvement of mineralocorticoids or decreased functional renal tissue in spontaneous hypertension.

METHODS

ANIMALS

Breeding Program

Rats were obtained from several sources. Surgical techniques were initially developed using 70 mixed-sex rats that were surplus from the Department of Medical Psychology, UOHSC. Additional surgical experience was gained with 82 SHR and WKY rats purchased from a commercial breeder (Laboratory Supply Co., Indianapolis, IN).

On August 19, 1977, I transferred SHR and WKY rats from a colony at the Oregon Regional Primate Center, Beaverton, Oregon and started a colony in the Animal Quarters, UOHSC. Dr. H. Uno has maintained the ORPRC colony from a stock of 4 week old SHR and WKY rats that were purchased from Laboratory Supply Co., in May 1977. Both ORPRC and UOHSC colonies have been maintained by brother-sister matings. Rat pups were weaned at the age of four weeks. Cages with SHR and WKY rats were kept on separate racks to minimize inadvertently mixing the strains.

One of the rats used to define the renal function curve in 8 to 12 week old WKY rats was obtained from the ORPRC colony in August 1978. All other observations for the renal function curves were made with rats from the UOHSC colony. SHR and WKY rats were at F39 to F44 and F17 to F22 from the NIH colony which was in turn developed with stock from the original Kyoto colony in 1967. My colony has produced a total of 287 SHR and 149 WKY rats. Unused rats were donated to several other research workers at the UOHSC at the termination of the experimental work described herein.

Care of Animals

All rats were maintained on tap water ad libitum and rat chow from Oregon State University. Flame photometric analysis revealed 0.14% Na and 0.4% K by weight for this chow. Purina Lab Chow^{R} has 0.49% Na and 0.82% K.

Before surgery, rats were housed without crowding in suspended metal cages in the Animal Care Quarters, UOHSC with a 12 hour light-dark cycle. Temperature was controlled at 21 C. After surgery, the rats were kept in individual cages within a flow-through hood in the laboratory. Although the laboratory was air-conditioned, temperature was not as rigidly controlled as it was in the Animal Care Quarters. The window blinds in the laboratory were kept open.

Weight gain of these rats (Figure 9, 10, 11 and 12) was similar to that which has been observed in other laboratories (Freis, Ragan, Pillsbury, et al., 1972; Iams and Wexler, 1977; Pfeffer, Ferrell, Pfeffer et al., 1978). By 14 to 19 weeks, the female WKY rats weighed more than the female SHR rats.

Figure 9: Body weight of female SHR rats. Weight was measured immediately before the surgical implantation of catheters. The curve is the least-squares fit for a quadratic equation.

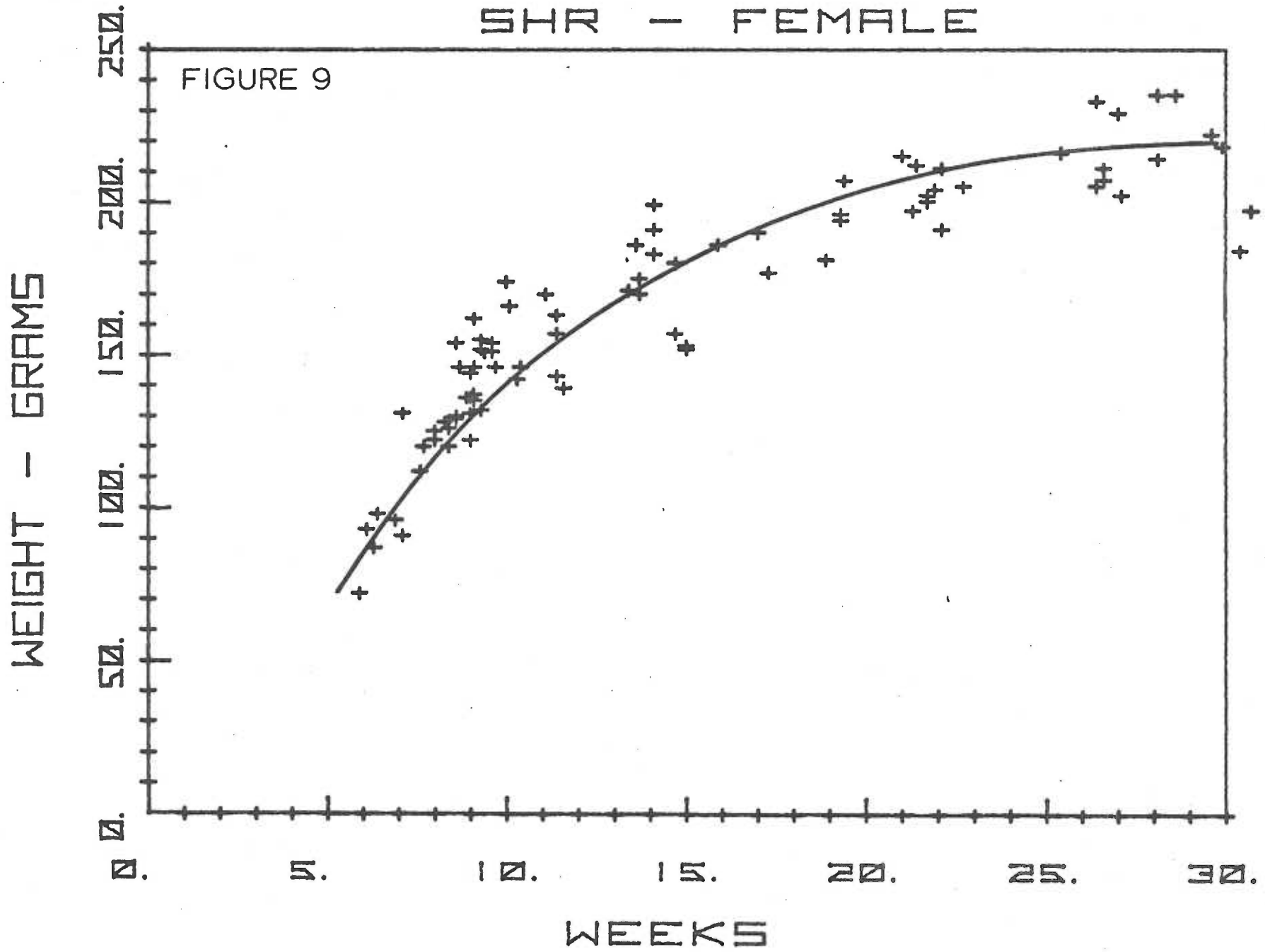

SHR - FEMALE
FIGURE 9
WEIGHT - GRAMS
250.
200.
150.
100.
50.
0.
0.
5.
10.
15.
20.
25.
30.
WEEKS

Figure 10: Body weight of female WKY rats. Weight was measured immediately before the surgical implantation of catheters. The curve is the least-squares fit for a quadratic equation.

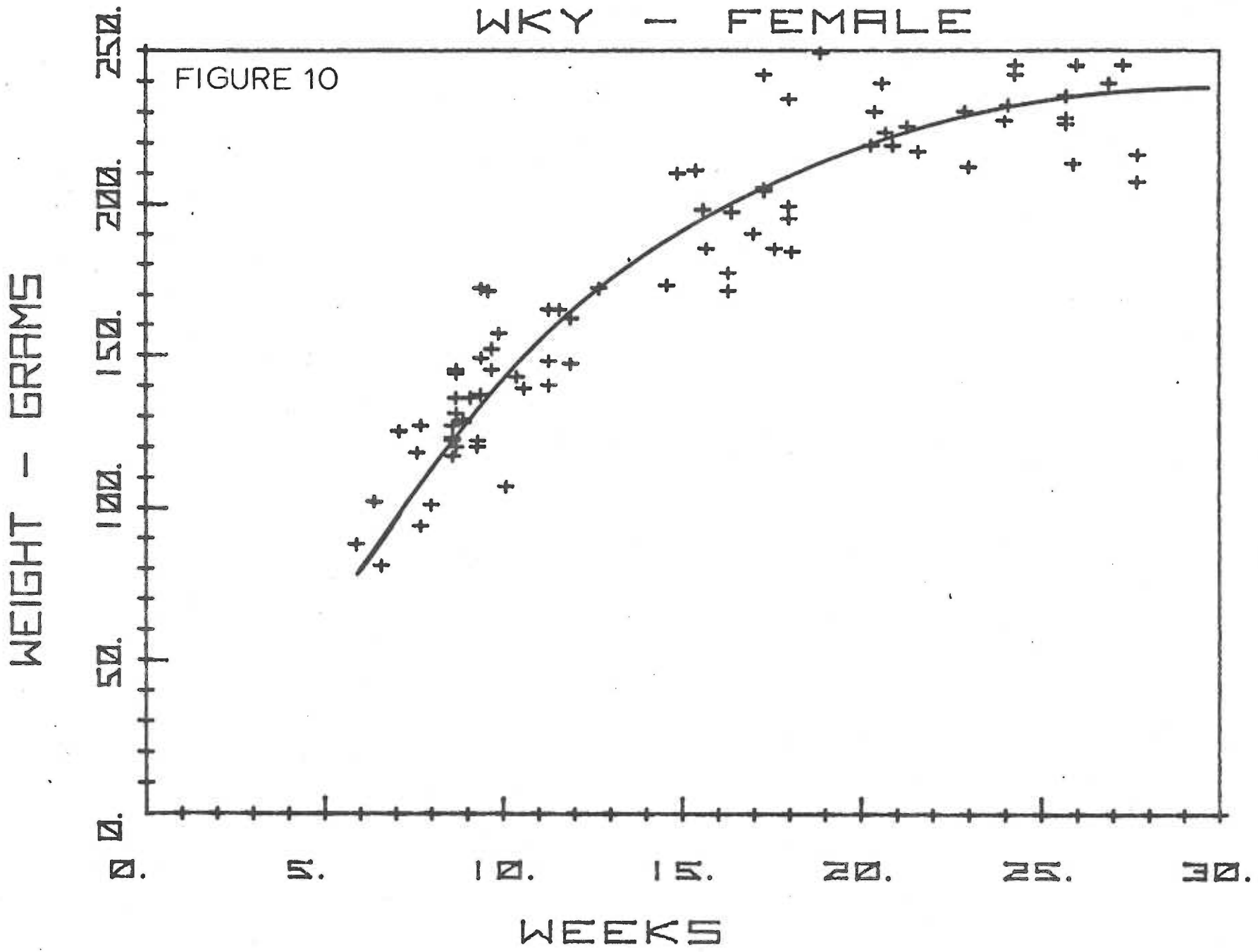

WKY – FEMALE
FIGURE 10
WEIGHT – GRAMS
250.
200.
150.
100.
50.
0.
0.
5.
10.
15.
20.
25.
30.
WEEKS

Figure 11: Body weight of male SHR rats. Weight was measured immediately before the surgical implantation of catheters. The curve is the least-squares fit for a quadratic equation.

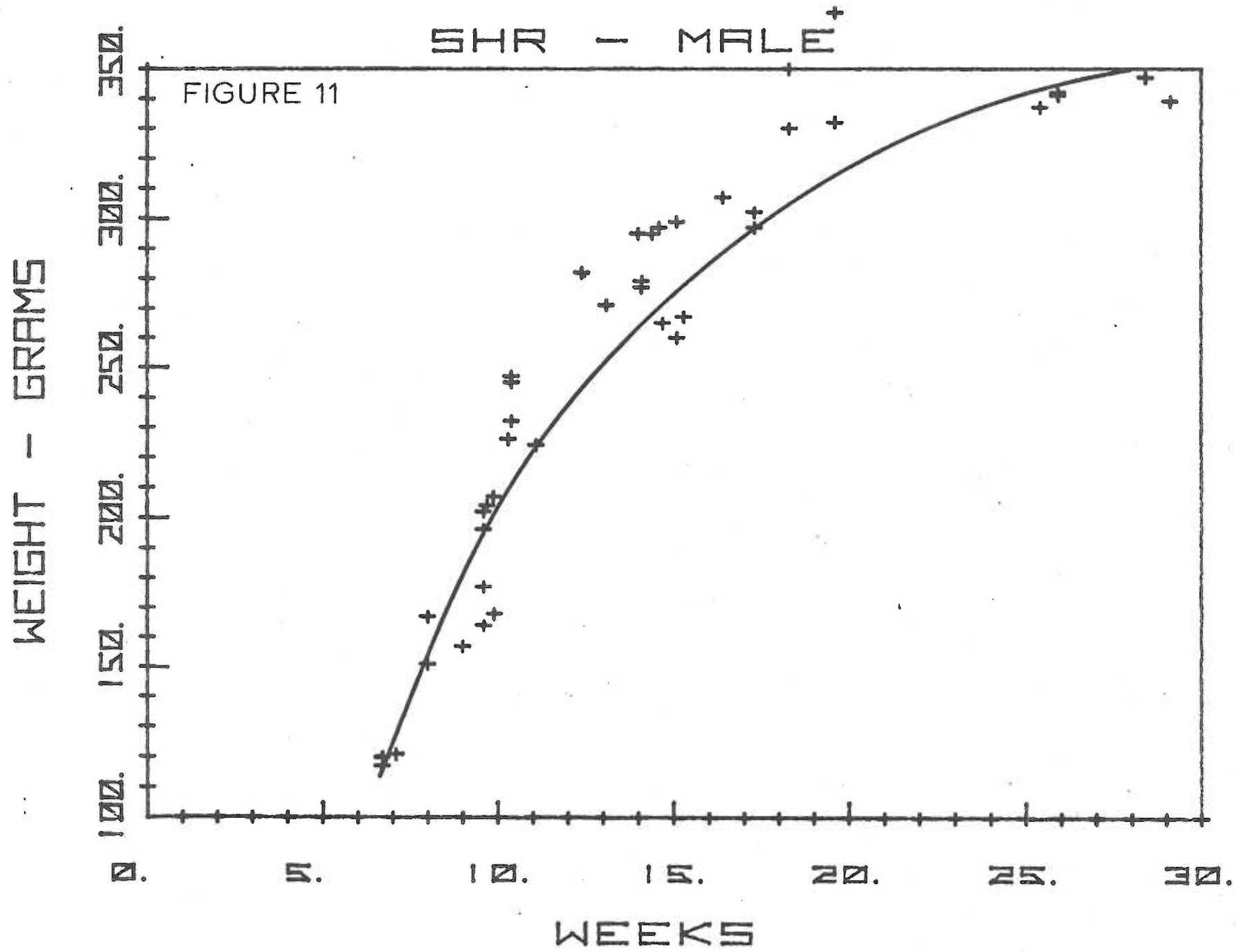
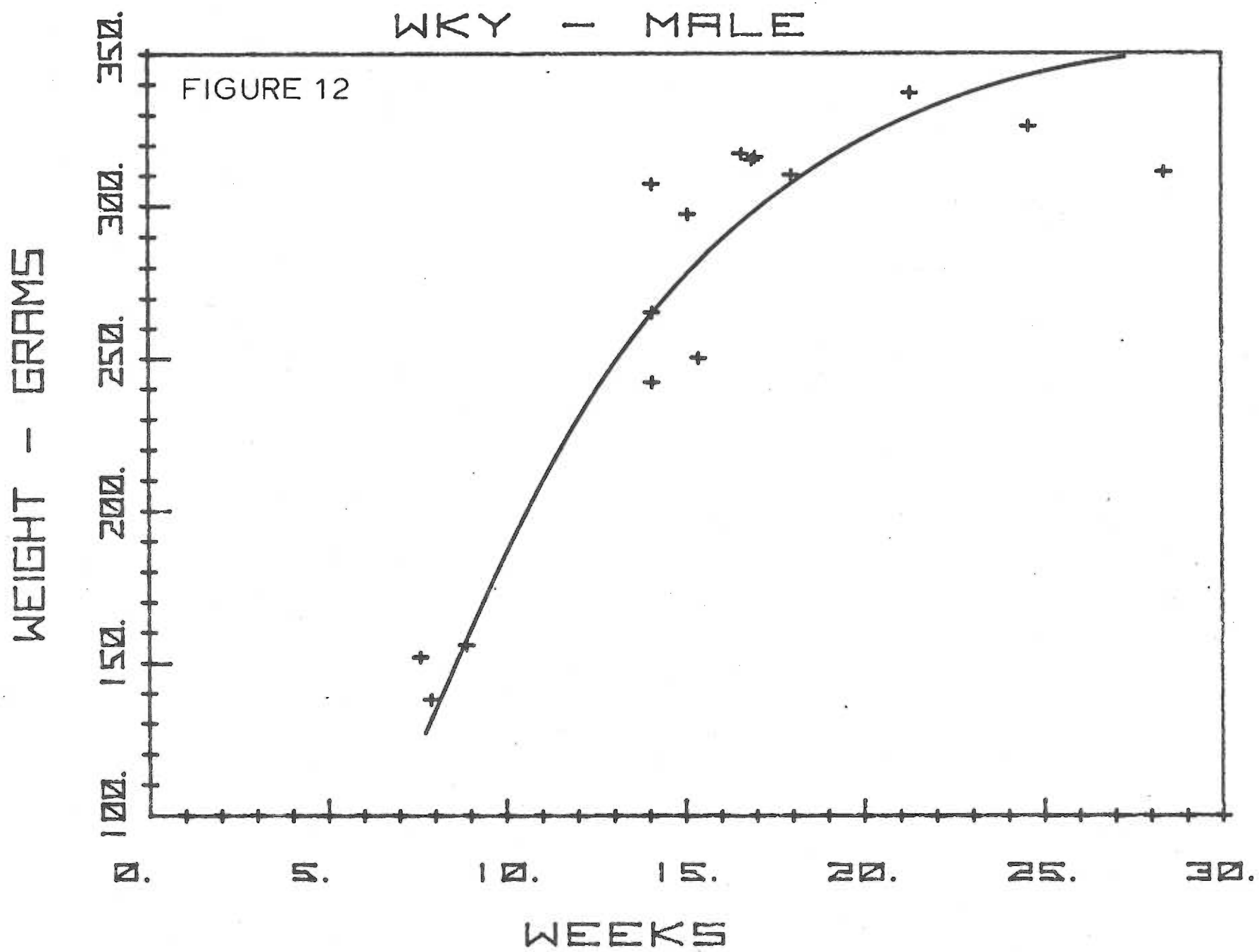

SHR - MALE
WEIGHT - GRAMS
100.
150.
200.
250.
300.
350.
0.
5.
10.
15.
20.
25.
30.
WEEKS

FIGURE 11

Figure 12: Body weight of male WKY rats. Weight was measured immediately before the surgical implantation of catheters. The curve is the least-squares fit for a quadratic equation.


WKY – MALE
FIGURE 12
WEIGHT - GRAMS
100.
150.
200.
250.
300.
350.
0.
5.
10.
15.
20.
25.
30.
WEEKS

FABRICATION OF CATHETERS

Catheters were fashioned using the techniques described by Heatley and Weeks (1964). Both polyethylene tubing (Dural Plastic, Dural, N.S.W., Australia) and silastic tubing (Dow-Corning, Midland, MI) were used. When two pieces of tubing were to be joined, a steel wire was first inserted into the lumens of the tubes. Next, a piece of shrinkable tubing was fitted over both pieces of tubing to strengthen and to seal the joint. When the shrinkable tubing was heated in a stream of heated air it would shrink to form a tight couple between the two pieces of tubing. In addition, silicone cement (Dow-Corning) was applied to the junction between silastic tubing and shrinkable tubing. Suture material was tied between small ridges on the polyethylene tubing. The ridges were made by heating a small area of the tubing in a stream of heated air with a wire in the tube to prevent closure of the lumen.

Loops of polyethylene catheters were formed by wrapping the catheter around a 4.5 mm diameter brass rod. The rod and loop assembly was dipped in boiling water for two seconds and then into cool water.

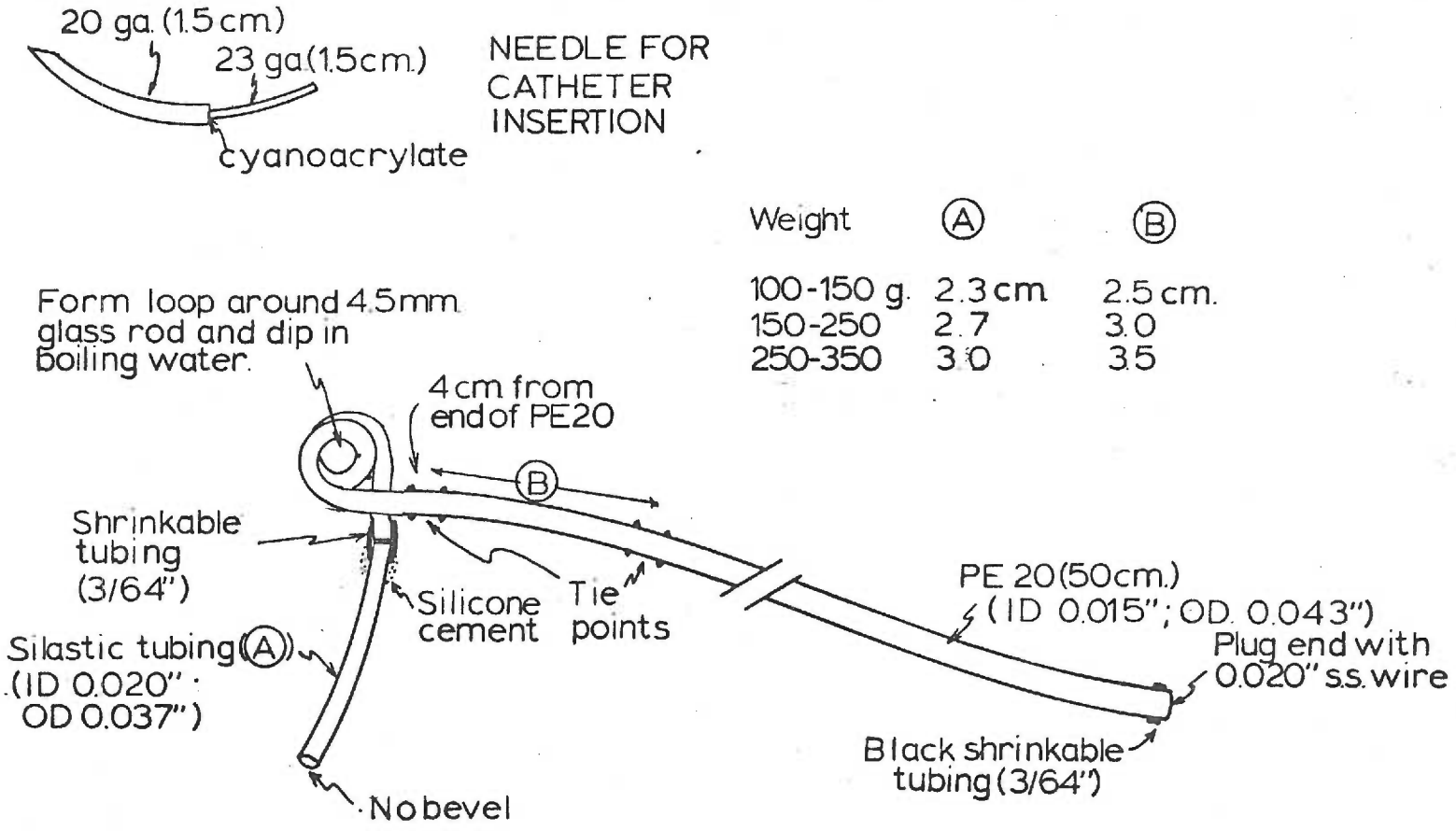
The end of the catheter that was to be inserted into the right atrium via the external jugular vein (Figure 13) was fashioned from a piece of silastic tubing. Silastic tubing was used in lieu of the stiffer polyethylene tubing which traumatizes the venous and atrial walls. The tip was cut without a bevel to prevent the collapse of the vein against the catheter opening.

A small-diameter piece of polyethylene tubing at the end of the aortic catheter (Figure 14) was necessary to prevent partial occlusion of the aorta in younger rats. A bevel cut at the tip of the catheter

enabled it to be inserted into the aorta. The loop placed in both the venous and the aortic catheters was designed to allow for free movement without pulling the catheter from the vessel.

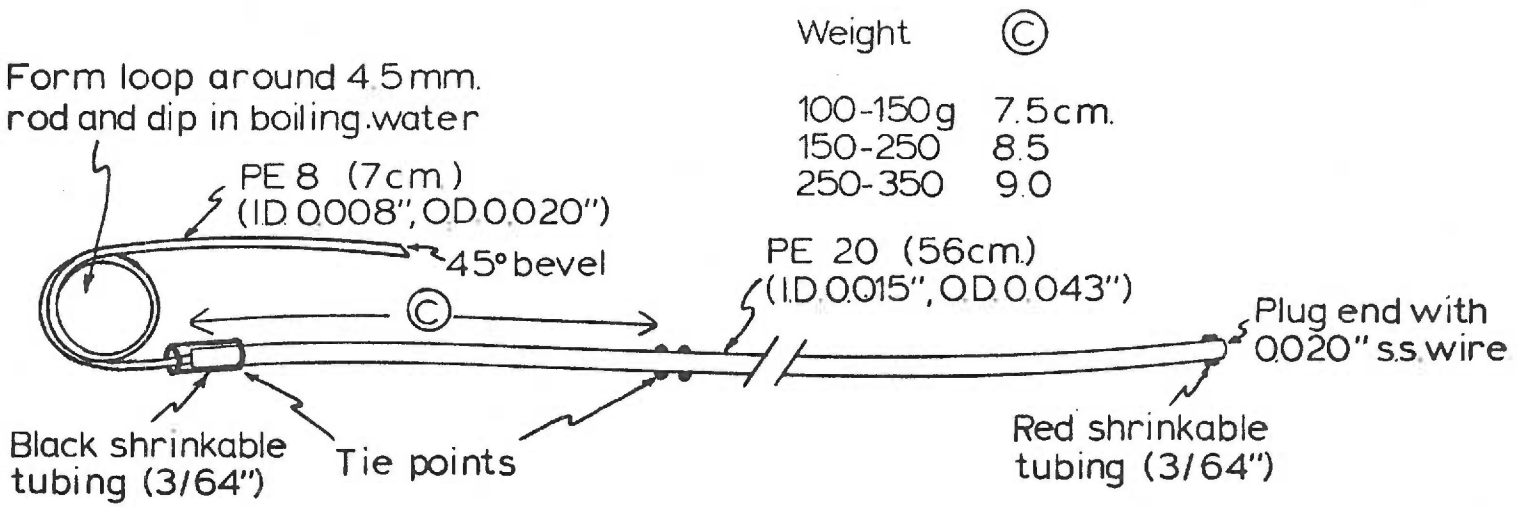
The bladder catheter (Figure 15) was formed from double-lumen polyethylene tubing so that the bladder could be readily flushed and emptied. A polyethylene ball on the end of the catheter allowed free collection of urine.

Figure 13: Procedures for the fabrication of the catheter which is designed to go through the external jugular vein so that the tip lies in the right atrium.



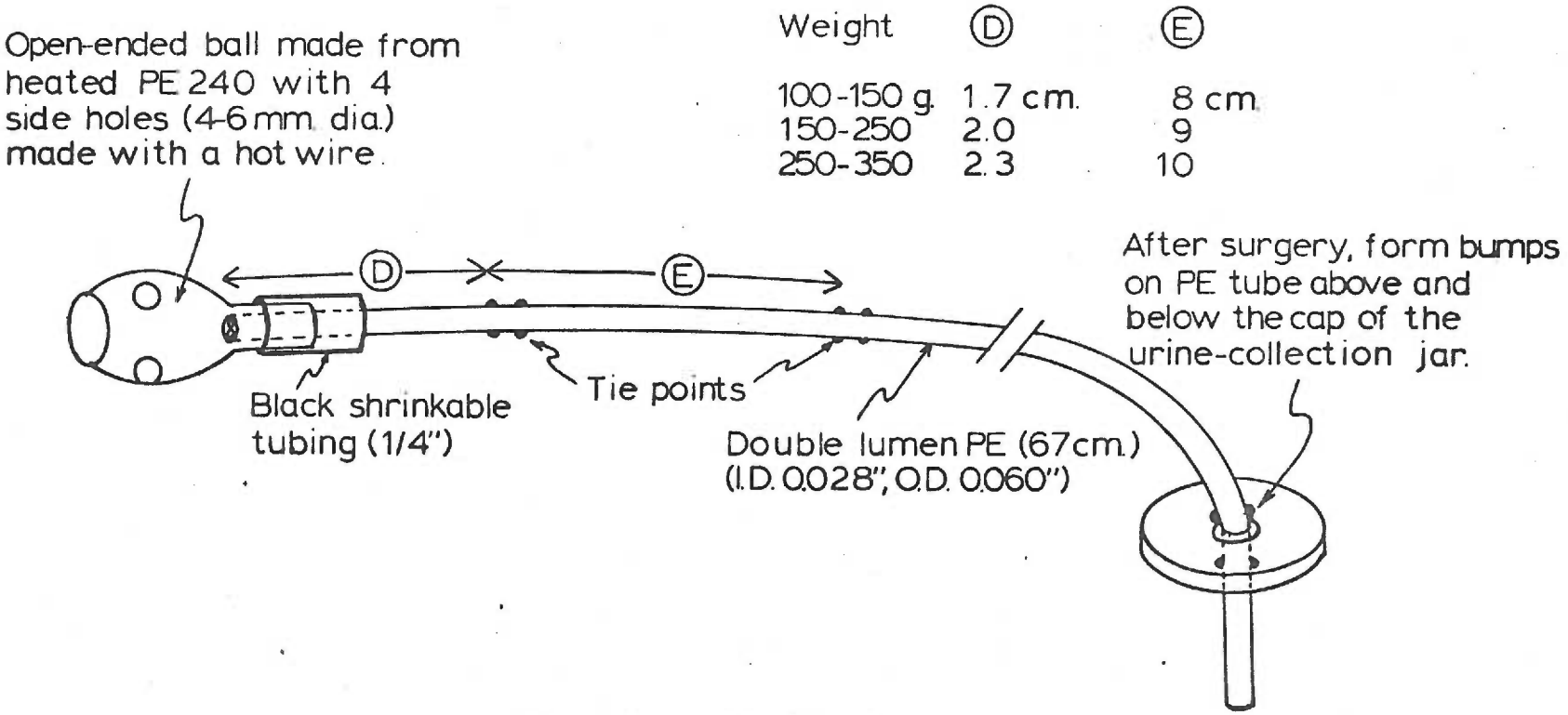
Jugular Catheter

Figure 14: Procedures for the fabrication of the catheter which was designed to be placed in the abdominal aorta.



Aortic Catheter

Figure 15: Procedures for the fabrication of the catheter which was designed to be placed in the urinary bladder.



Bladder Catheter

SURGERY

General Surgical Procedures

I wore a mask and cap but no gloves while operating on the rats. Instruments were immersed for at least 30 minutes before use in a 1:750 dilution of 17% benzalkonium chloride (Zephiran^{R}, Winthrop Labs, NYC). Catheters and harness springs were stored in 70% alcohol. The incision area was shaved and swabbed with iodine solution (Betadine solution^{R}, Purdue-Frederick Co., Norwalk, CN). Two to three hours of surgical anesthesia was produced with 3.5 to 4.5 mg/100g pentobarbital i.p. (Pentosol^{R}, Burns Biotec, Oakland, CA). If I was careful to use no more pentobarbital than was necessary, the rat's airways remained open without the aid of tracheal intubation. Ketamine (Ketalar^{R}, Parke-Davis, Detroit, MI), 0.5 mg/100g i.v. was used as a short-term anesthetic when it was necessary to reattach the harness spring to the back of the neck. Penicillin (Pfizerpen^{R}, Pfizer Laboratories, NYC), 25,000 units i.m., was injected at the end of surgery. During surgery, adhesive tape around the paws secured the rat to an inverted metal tray that rested on a heating pad. All suture material was 4-0 silk (Ethicon Inc., Somerville, NJ). Eleven mm wound clips (Propper. Long Island City, NY) served to close the skin.

I modified the venous and arterial catheterization methods from those of Weeks (Weeks and Jones, 1960; Weeks and Davis, 1964). I developed a new method for catheterizing the rat urinary bladder after adopting suggestions made by Michael Boileau, M.D. (Division of Urology, UOHSC). Figure 16 is a graphical summary of the catheterization procedures.

Venous Catheter

A catheter was placed in the external jugular vein so that its tip lay in or near the right atrium. After making a 2 cm incision to the right of the midline (the center of which was just above the right clavicle), I formed a pocket under the submaxillary gland and exposed the right external jugular vein (Figure 16). A sufficient length of vein was dissected from surrounding tissue in order to place a single ligature about 5 mm rostral to the edge of the pectoral muscle. Another suture was passed around the vein near the edge of the muscle. A trocar, which was fashioned from 13 ga. stainless steel hypodermic tubing (Small Parts, Miami, FL) and a pointed brass tip, was used to pull the catheter through the right sternomastoid muscle to a 2 cm longitudinal incision which had been made between the scapulae. After testing the patency of the catheter and filling it with 0.9% sterile saline, I sutured the loop of the catheter to the sternomastoid muscle.

To minimize bleeding from the jugular vein during catheterization, I used the following techniques (Herd and Barger, 1964): I first joined sections of 20 ga. and 23 ga. stainless steel tubing to form a curved needle (shown in Figure 13). After fitting the silastic tip of the jugular catheter over the smaller portion of the curved needle, I then passed the needle into the external jugular vein just proximal to the pectoral muscle above the clavicle and then out through the muscle about 5 to 10 mm caudal to the muscle's rostral edge. By then grasping the silastic tubing over the needle, I was able to push the silastic tube through the vein and out through the muscle. After removing the needle, I pulled the catheter back into the jugular vein before advancing it

into the vein until the shrinkable tubing stopped against the vein. I ligated the catheter in place using both of the ligatures which I had previously placed around the vein. I then collected 0.5 ml of blood in heparin for the determination of pre-surgical plasma creatinine concentration. After filling the catheter with a solution of polyvinylpyrrolidone (PVP) and heparin in saline, I inserted a piece of 0.020" stainless steel wire to close the lumen. After I tucked the catheter loop in the pocket under the submaxillary gland, I used wound clips to secure the everted and apposed edges of the skin. In those animals which were programmed for blood donation, the final step was to attach a spring to the back of the neck. In female rats which were programmed for studies of the renal function curve, I also catheterized the aorta and urinary bladder.

Aortic Catheter

A second catheter was placed in the abdominal aorta just above the iliac bifurcation. A 5 cm midline abdominal incision extending to a point just rostral to the external urethra was held open with a self-retaining retractor to permit access both to the abdominal aorta and to the bladder. After moving the intestines to the right of the aorta and opening the posterior peritonium between the genito-femoral nerve and the left ureter, I advanced an 8 ga. stainless steel trocar with a pointed brass tip through the psoas muscle to the subcutaneous fascia of the back and thence to the incision at the back of the neck, taking care to avoid the vertebrae and the diaphragm. The aortic and bladder catheters were pulled through with the trocar and attached to the psoas muscles where they passed through it. I was careful to avoid the near-by left ureter. When the aortic catheter had been positioned so that the loop lay flat and the straight part lay along the aorta, the end of the catheter was trimmed at about a 45 degree bevel so that the tip was at least 1 cm below the renal arteries. Patency of the aortic catheter was verified upon filling it with 0.9% sterile saline.

I used a cotton swab to clear loose connective tissue from about 5 mm of the ventral surface of the aorta above the iliac bifurcation. With the tip of my left ring finger, I applied pressure to the aorta just caudal to the renal arteries to stop blood flow while I punctured the aorta just rostral to the bifurcation with a bent 25 ga. needle held in my right hand. Withdrawal of the needle left a hole in the aorta that was just large enough to accept the small PE 8 (0.008" O.D.) end of the aortic catheter. The catheter was advanced upstream until there

was no tension on the loop. It was not necessary to attach the catheter to the aorta. There was no bleeding around the catheter where it entered the aorta and the loop in the catheter prevented the catheter from being pulled out when the rat moved. Since I would occasionally put the catheter through the aorta into the vena cava, I verified that the catheter was in the aorta by checking for the presence of a spontaneous flow of fluid from the external end of the catheter. I filled the catheter with PVP-heparin-saline and closed it with a piece of 0.020" stainless steel wire. It was important to pinch tightly the external end of the catheter after filling it with PVP-heparin-saline and before inserting the piece of wire. If any back-flow into the catheter was allowed, a clot was certain to form within an hour.

Bladder Catheter

The urethra was tied as near to the pubic bone as possible to avoid the ureteral obstruction that would occur when I made the ligation near the bladder. I opened the venous plexus between the vagina and urethra so that I could pass a suture around the urethra. There was a loss of blood, sometimes as much as 0.5 ml, for a minute or so after I opened the plexus.

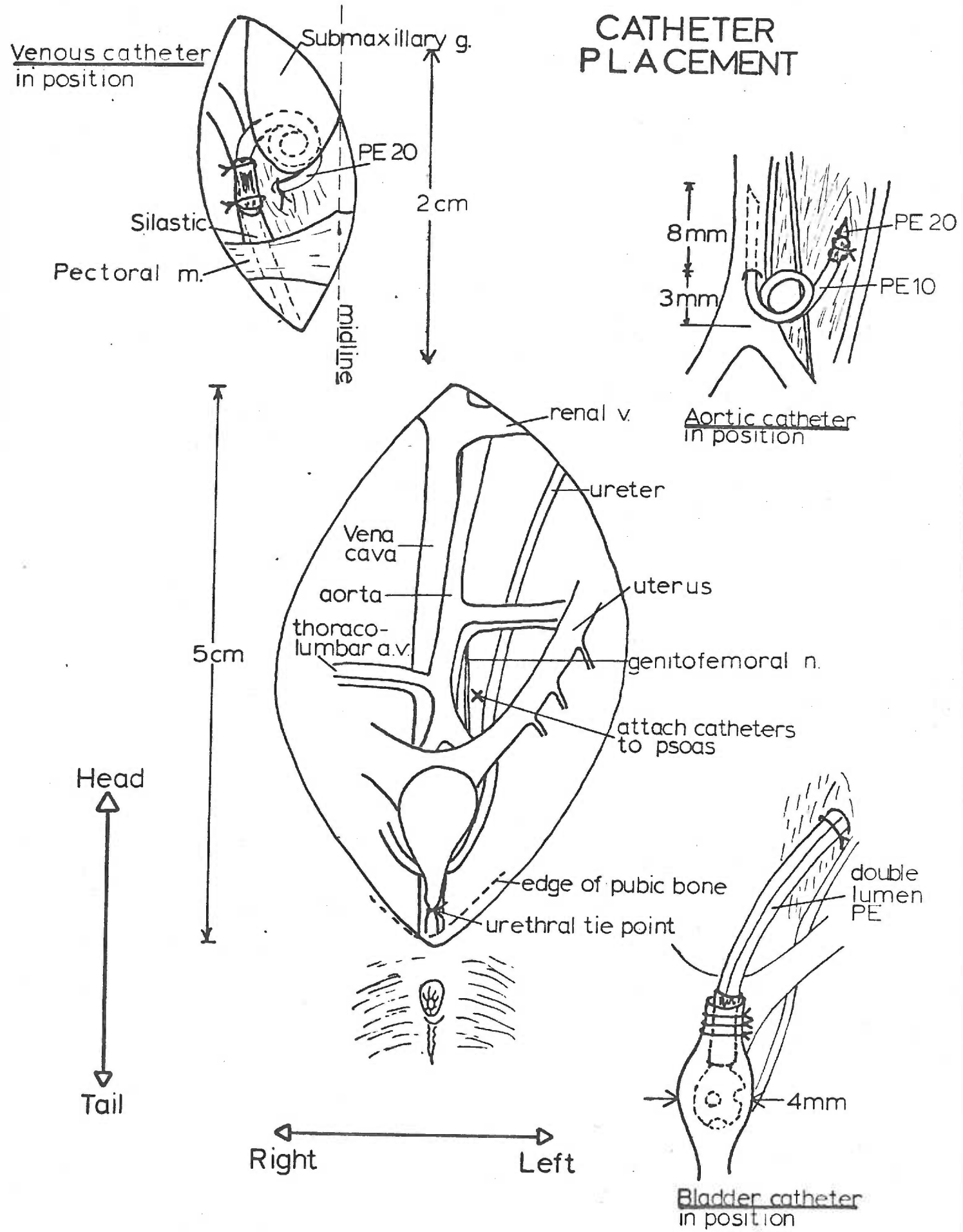
The bladder catheter was sutured to the psoas alongside the aortic catheter so that the ball on the end of the catheter would fit within the bladder without stretching it. After a purse string suture had been run around the equator of the bladder, the dome of the bladder was excised, allowing me to insert the ball-shaped end of the catheter into the bladder. I secured the catheter in the bladder with the purse string suture by wrapping the suture two or three times about the shrinkable tubing which joined the ball to the rest of the bladder catheter. Each loop of the suture was placed a bit closer to the catheter ball than was the previous loop.

By rapidly flushing about 2 ml of 1/4% acetic acid (vol./vol.) through the catheter and bladder, I could verify that there were no leaks from the bladder.

I closed the abdomen in two stages. First, the rectus muscles were apposed with a simple continuous lock stitch (Lang, 1976). Then the skin was closed with wound clips.

Figure 16: Description of the surgical placement of the jugular, aortic and bladder catheters.

CATHETER PLACEMENT



Harness and Cage

Attachment and protection of the catheters in the minimally-restrained rat was by a method adapted from Cox and Beazley (1975). All catheters leaving the back of the neck were individually attached to the neck muscles before they were run through the stainless steel spring which is shown in Figure 17. This spring was sutured to the neck muscles by a plastic disc which was attached with cyanoacrylate glue (Super-Glue 3^{R}, Loctite Corp., Cleveland, OH) to the spring. Wound clips were used to close the skin over the plastic disc and bacitracin ointment (Baciguent^{R}, Upjohn Co., Kalamazoo, MI) was applied to the wound. This was the only one of the three wounds which had a tendency to abscess; this could be a result of the spring which provided an open route for bacteria to continually seed the subcutaneous tissue.

After surgery, the rats were placed in standard metal cages (17.5 cm wide x 24 cm deep x 18 cm high) with base and top fashioned from opaque acrylic plastic (Figures 18 and 19). Table 6 specifies the pieces required to make the base and top. I glued the plastic with chloroform. The base and top were designed to allow the cages to be stacked double. Male blood donors with venous catheters only were kept in the lower cages and female rats with aortic, venous and bladder catheters occupied the second tier.

Catheters and spring were passed first through a hole in the cage top and then through a hole in a 1" plastic ball. Adhesive tape fixed the spring to the ball, thus allowing the rat free movement within the cage. Multiple catheters were bound together with two or three pieces of adhesive tape. The bladder catheter was secured in a hole on the top

of the urine collection bottle by heating the catheter while wires were in the lumens to form bulges just above and below the bottle top. Several milliliters of toluene were placed in the urine bottle to minimize evaporative loss of urine and as a bacteriostatic agent. The animal had access to food and tap water after surgery.

Animals were left as shown in Figure 20. The spring and ball assembly permitted the rat to move around the cage relatively freely. They were not permitted to roll over, but this behavior was not observed in untethered caged rats. For the rest of their lives they were neither anesthetized nor restrained except on those occasions when it was necessary to administer ketamine in order to repair the attachment of the spring at the back of the neck.

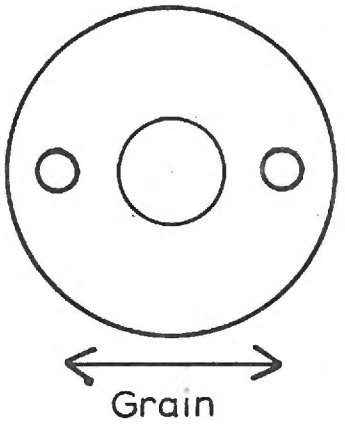
Figure 17: Fabrication of the catheter harness and the placement of the harness through the top of the cage.

HARNESS

Spring: 0.022" SS wire; 5/32" ID; 8" length

Plate: "Shrinky-Dink" cut to this template

Ball: Nylon; 1" dia.; 0.228" dia. hole



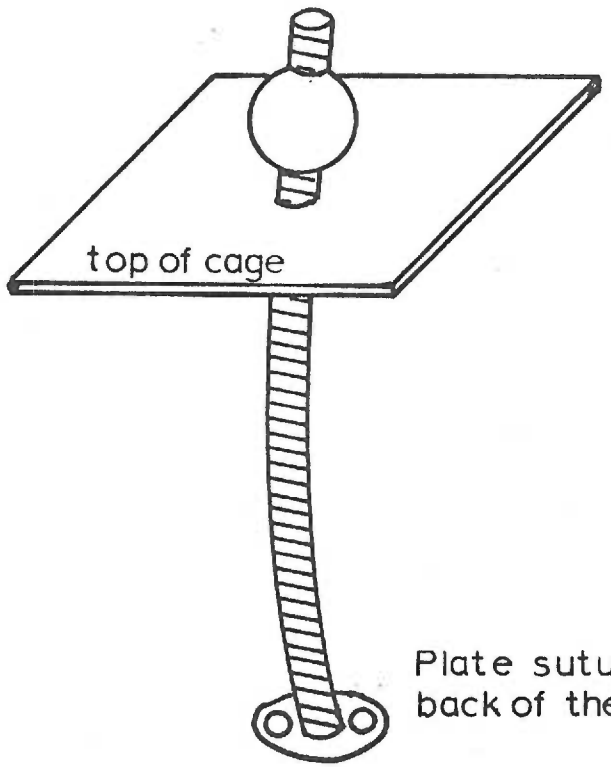
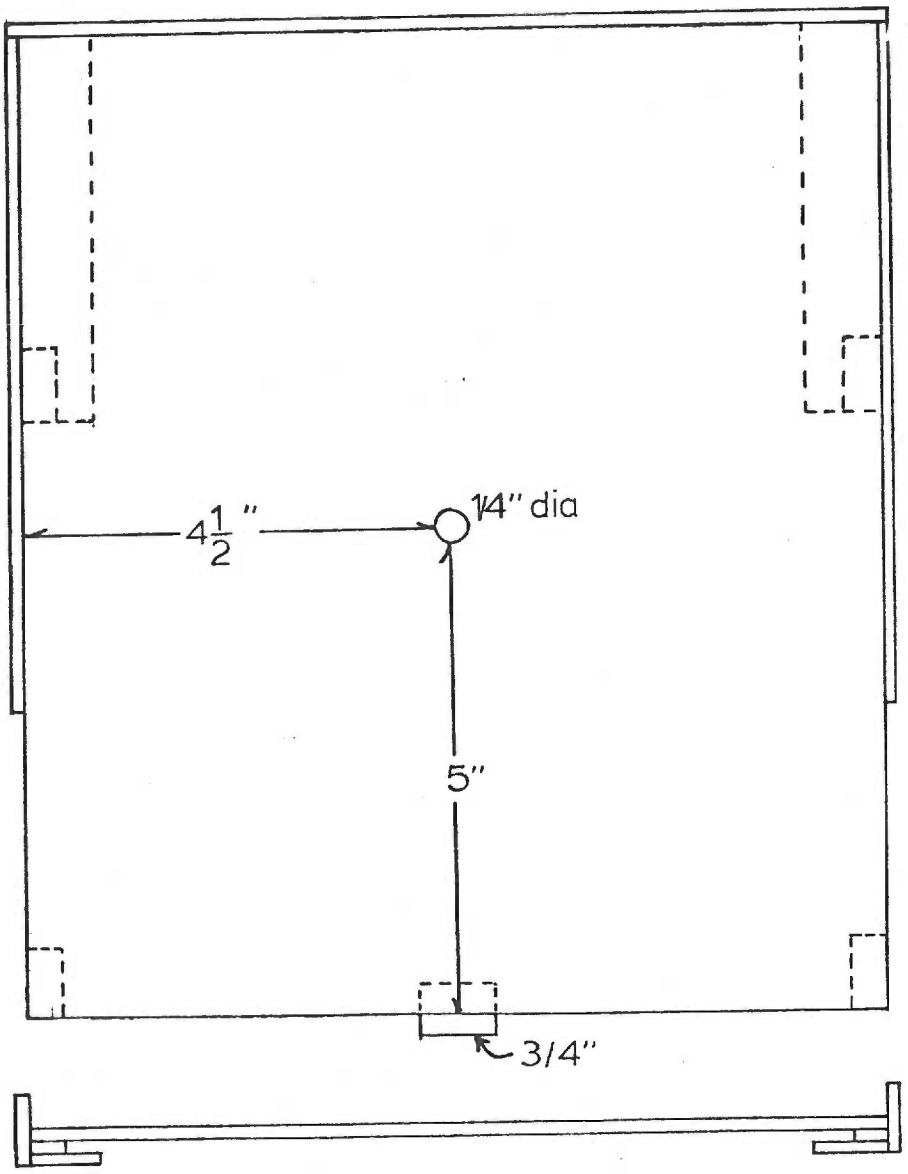


Figure 18: Fabrication of the top for the rat cage from 1/4 inch thick acrylic plastic. Scale is given in inches.



TOP

Figure 19: Fabrication of the bottom for the rat cage from 1/4 inch thick acrylic plastic. Scale is given in inches.

1 2 3"

BASE

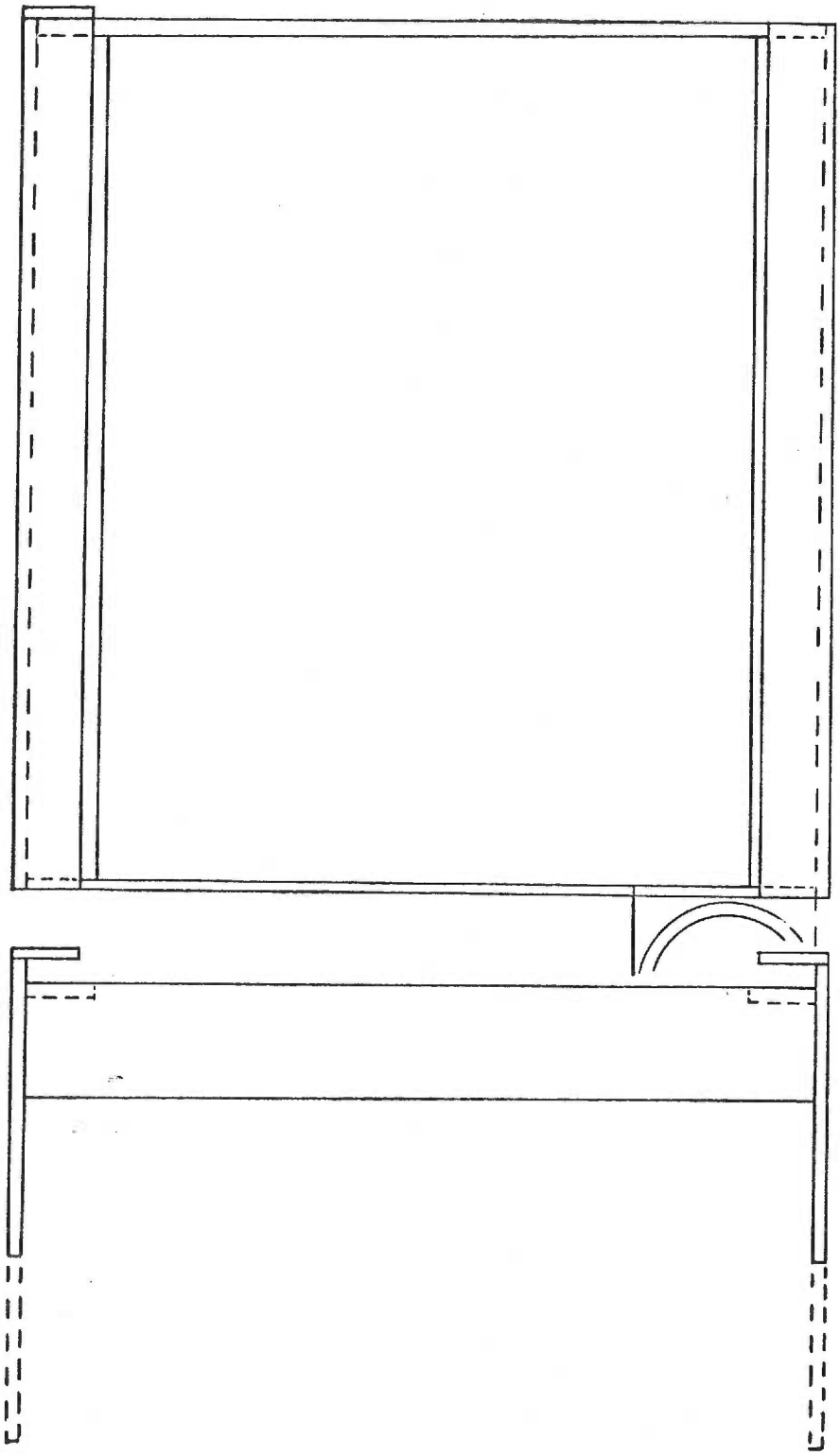
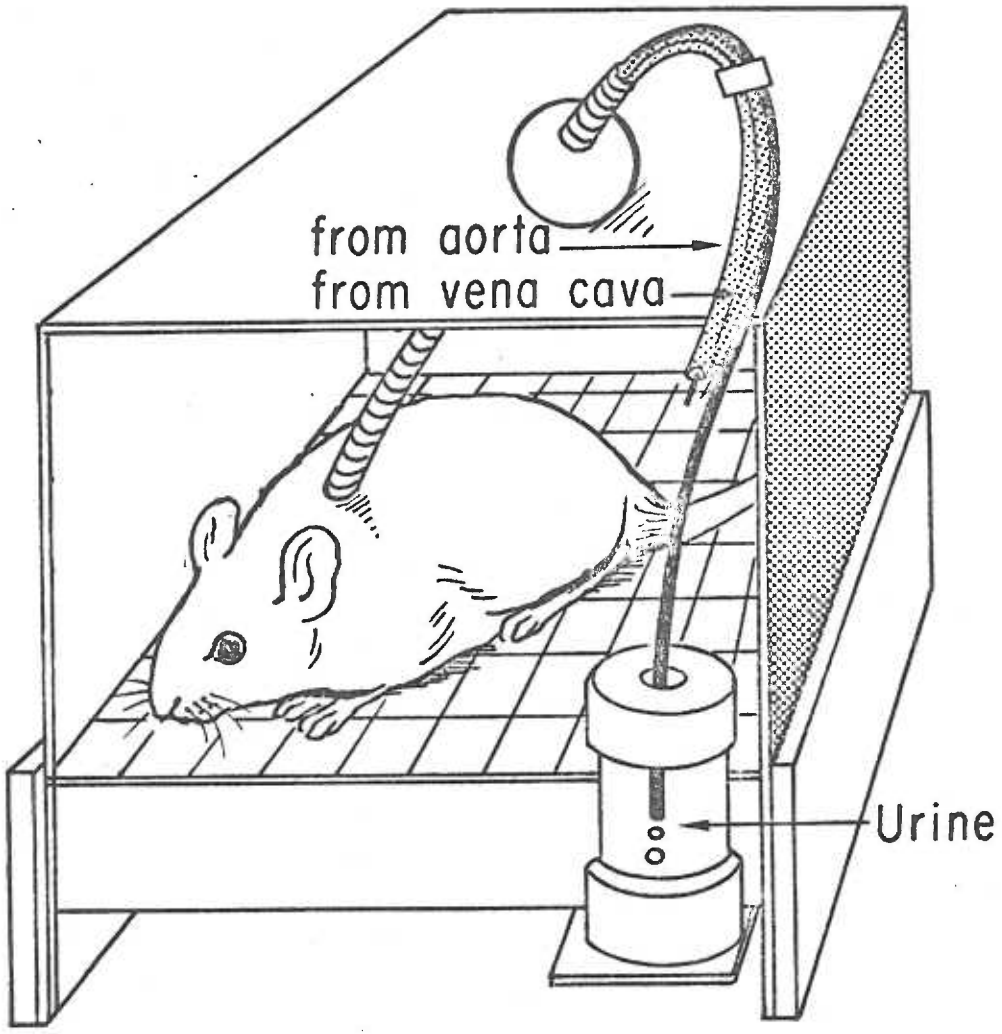


Table 6: Description of pieces of 1/4 inch acrylic plastic required for the base and top of the cages. Measurements in parentheses are for upper cages.

BASE		TOP	
Quantity	Size (inches)	Quantity	Size (inches)
1	(8 5/8 x 11 1/8)	1	8 7/8 x 10 1/8
2	9 1/2 x 3 1/4 (5 1/4)	1	9 1/8 x 3/4
2	3/4 x 9 1/2	2	7 x 3/4
2	3/4 x 9 1/4	5	3/8 x 3/4
2	1 1/4 x 8 5/8	1	3/4 x 3/4
1	2 7/8 OD x 2 *	2	4 x 3/4

* Piece of tubular plastic as the holder for the urine collection bottle.

Figure 20: The appearance of the rat in a cage. The front of the cage has been cut away to better view the rat and the attached spring harness.


from aorta
from vena cava
Urine

ACUTE EXPERIMENTS

General Procedures

The relation of sodium excretion rate to mean arterial pressure in conscious, unrestrained rats with ganglionic blockade was determined in acute experiments. These experiments were carried out at least 48 hours after surgery and between 3 PM and 9 PM. It was often possible to change the mean arterial pressure during the second of two urine collection periods by either infusion of fresh whole blood or by bleeding the rat. There were never more than two urinary collection periods per day for any rat.

Aortic and venous catheters were first cleared with 0.9% sterile saline. Then the aortic catheter was connected to a pressure transducer (23Gc, Statham Instruments, Hato Rey, Puerto Rico) by a 23 ga. needle. With this catheter mean arterial pressure was continuously recorded during experimental periods. Arterial pulsations could often be used to measure the pulse rate but the fidelity of the system did not allow me to measure consistently the systolic and diastolic pressures. The venous catheter was attached to a pressure transducer (Statham 23 AC) and one lumen of the bladder catheter was connected to a syringe with 1/4% acetic acid. Figure 21 shows the relation of catheters and transducers.

After recording basal blood pressure for several minutes, I injected 2 mg/100g hexamethonium bromide i.v. (Sigma Chemical Co., St. Louis, MO) which caused ganglionic blockade and hypotension within 30 seconds. Every two minutes for the rest of the experimental period, I injected smaller amounts of hexamethonium (0.5 mg/100g). Figure 22

illustrates the injection schedule and other temporal relations in acute experiments. After the initial hexamethonium injection, I optionally withdrew blood or infused fresh whole blood or did neither. Blood for infusion was obtained from conscious age- and strain-matched male rats via an indwelling jugular catheter. No more than 1.5 ml/100g BW of blood was taken from the donor rat. I had previously found that plasma renin activity of donor blood was elevated if more than this amount was withdrawn. It was usually possible to return an equivalent amount of blood from the experimental rat to the donor rat at the end of the experiment. I used acid-citrate-dextrose (ACD) as an anticoagulant until the blood was injected into the experimental rat. When the mean arterial pressure had stabilized I injected 3.5 ml of 1/4% acetic acid into one lumen of the bladder catheter and discarded the fluid that was thereby flushed from the bladder. I immediately placed the end of the bladder catheter into a 3 ml graduated cylinder and started the 10 minute urine-collection period. The bladder was again flushed with 2.5 ml of 1/4% acetic acid at the end of the collection period.

If there was to be a second collection period, I either withdrew or infused blood so that the mean arterial pressure decreased or increased during the second period relative to the first period. The procedures for collecting fluid from the bladder were the same for the second period as for the first urinary collection period.

Figure 21: Schema of the experimental apparatus.

Schematic of Experimental Apparatus

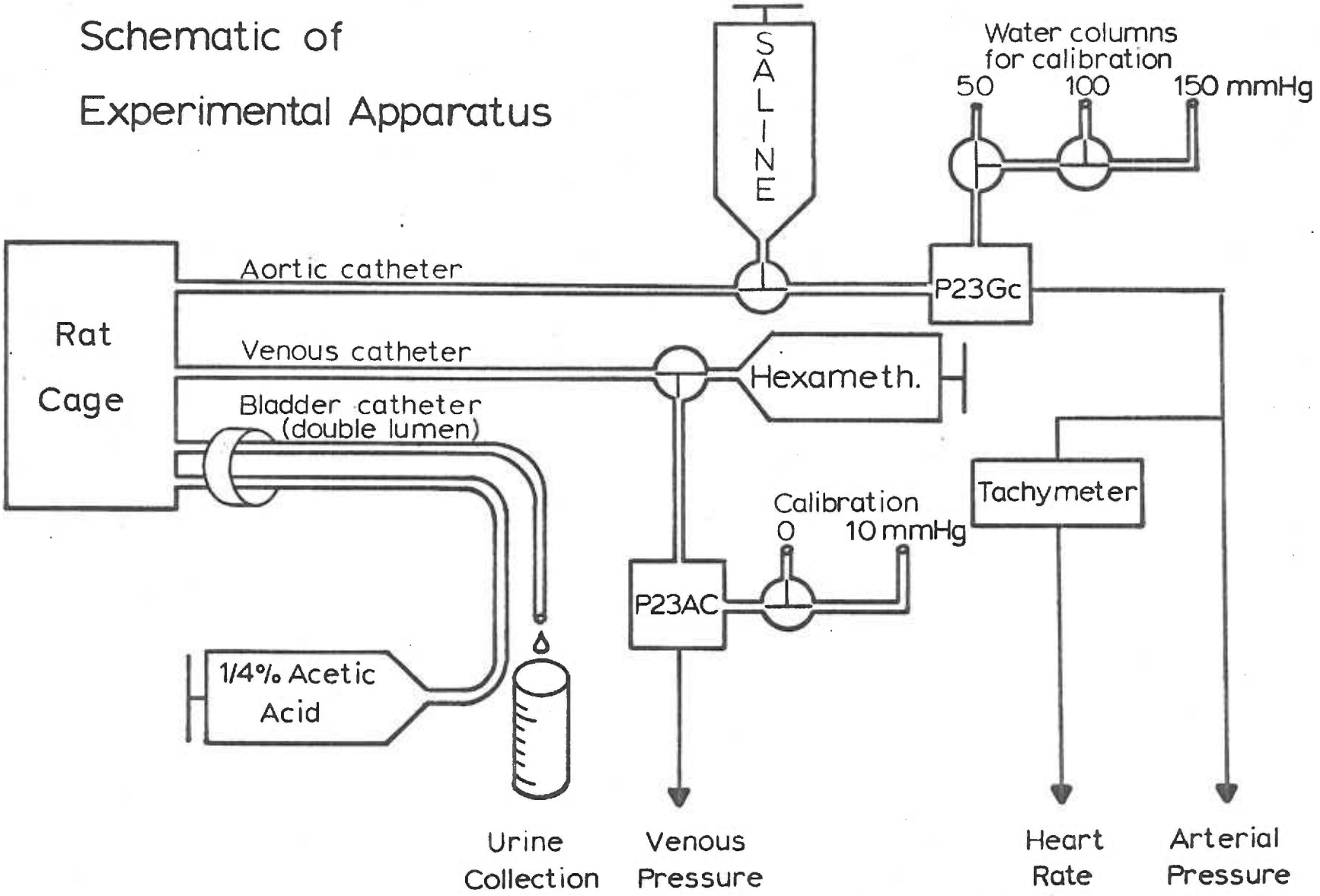
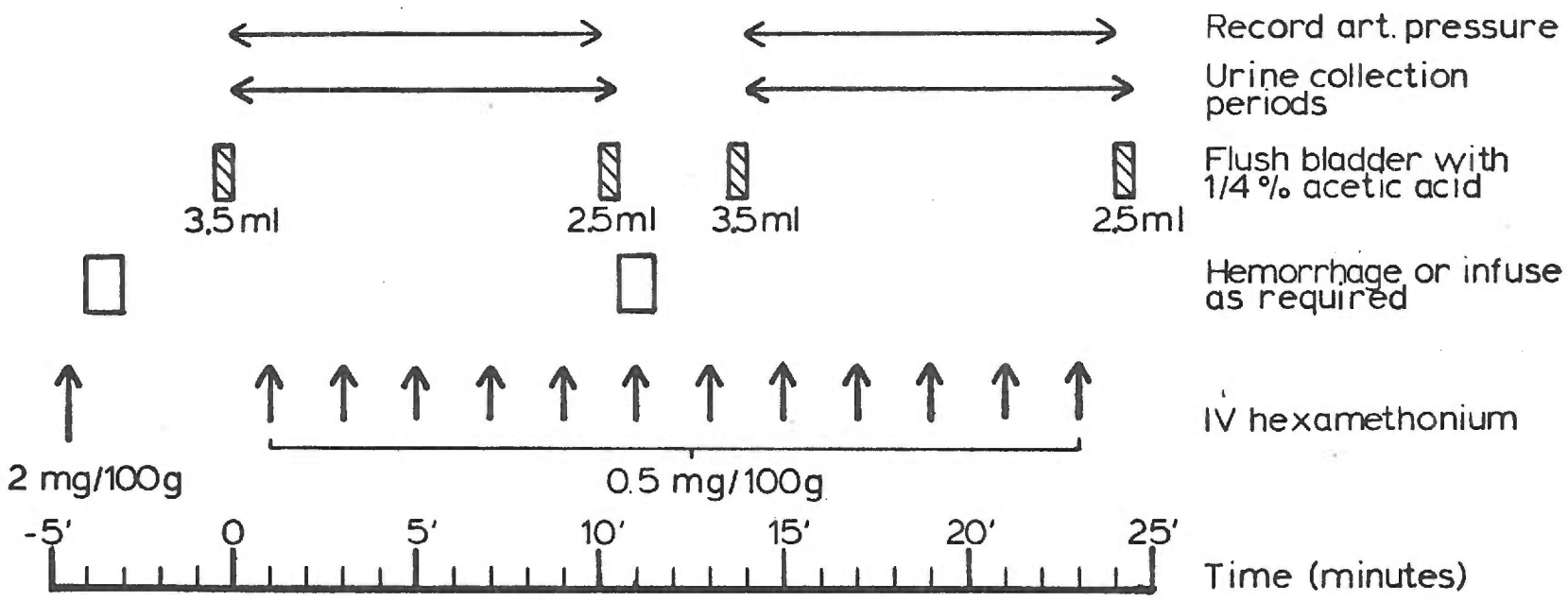


Figure 22: Summary of the protocol for obtaining data to be used in describing the renal function curves after ganglionic blockade with hexamethonium.

PROTOCOL

Renal Function Curves after Pharmacologic Denervation



Urine Collection

All sodium excretion rates are expressed as $mEq.min^{-1} \cdot 100\ g\ BW^{-1}$.

--Daily collection--

Urine drained from the rats into capped 70 ml bottles. Every one or two days, the bottles were emptied, urine volume was measured and aliquots were taken for later determination of $[Na^+]$ and $[K^+]$ or creatinine concentration. Several ml of toluene were added to the bottle before the cap was replaced.

At about 5 day intervals, I collected 0.5 ml of venous blood from experimental rats for later determination of plasma creatinine concentration.

I was therefore able to monitor daily excretion rates of Na^+, K^+, H_2O, and creatinine as well as periodic creatinine clearance rates.

--Acute experiments--

I wanted to collect urine samples in order to measure the sodium excretion rate during the experimental periods. Using the volume for the nephron plus collecting system obtained from stop-flow experiments (Harvey and Malvin, 1966), I have calculated that it takes 3 minutes for fluid to flow from the proximal nephron through the ureter. For this reason, I waited about three minutes after the injection of hexamethonium before I flushed the bladder with 3.5 ml of 1/4% acetic acid. The urine collection period began at the end of this first flush. The flush before the urine collection period effectively removed all sodium from the bladder so that the amount of sodium collected with the second 2.5 ml acetic acid flush was closely related to the renal sodium excretion rate for the 10 minute period between the first and second flush.

Using the measured resistance of one lumen of the bladder catheter, I calculated that the bladder pressure was about 20 mmHg while I was flushing the bladder at the rate of 2 ml/min.

I collected urine from the end of the first flush to the end of the second flush in a 3 ml graduated collection tube. After I measured the volume, I mixed the fluid by inverting the tube. I diluted three 100 microliter aliquots each in 5 ml of 200 ppm lithium ion solution (1:50). The samples were stored in Pyrex^{R} tubes at 4 C for up to 2 weeks before I measured the concentration of sodium and potassium ions by flame photometry.

Average Arterial Pressure

I began to measure mean arterial pressure at the end of the first bladder flush (3.5 ml). At this time, the bladder catheter was also placed in the collection tube. Since ureteral flow would likely be stopped while the bladder was being flushed, I chose to stop recording the arterial pressure after 10 minutes with the start of the second flush (Figure 22). I have not corrected for the estimated 3 minute delay between a change in glomerular capillary blood flow or filtration rate and a consequent change in ureteral sodium excretion rate (Harvey and Malvin, 1966).

The average arterial pressure during this period was obtained by taking the mean of 20 values taken 30 seconds apart which coincided with the 5 mm vertical lines on the recorder paper at 10 mm/min. The mean arterial pressure was not corrected to account for the fact that the catheter tip was pointing upstream in the aorta. I measured an internal radius of 0.06 cm for the aorta of a rat in which I estimated the cardiac output to be 29 ml/min. I therefore calculate that dynamic pressure would have added only 0.7 mmHg to the recorded pressure (Milnor, 1974).

It was often the case that arterial pressure pulses were damped out because the resistance of the catheter was high. Mean arterial pressure could then be read directly from the pressure record. In the event that arterial pressure pulsations were present on the recording, I was able to use the pulse count to measure heart rate but I could not directly read mean arterial pressure.

When I used the polygraph amplifier to electronically damp pulsatile arterial pressure recordings, I found this relation between the electroni-

cally damped mean arterial pressure (MAP), the apparent diatolic blood pressure (BPd') and the apparent pulse pressure (PP') to be:

$$MAP = BPd' + (0.50 \pm 0.03\ SEM)\ PP'$$

Thus, I estimated the mean arterial pressure in pulsatile recordings to be midway between the apparent diastolic and systolic pressures.

Criteria for Valid Data

A major objection to my results could be that, by introducing a catheter into the rat bladder, I was producing a situation likely to lead to pyelonephritis. In an effort to exclude from analysis data from those rats with impaired renal function, I eliminated experiments which met any one of these criteria:

1) Blood grossly visible in the urine.
2) Plasma creatinine concentration more than twice the value which was measured at the time of surgery.
3) Grossly-evident pyelonephritis or dilated ureters at post-mortem examination.

Some of the data from those rats with pyelonephritis apparent at post-mortem were used to define the renal function curves if the data were obtained before the onset of pyelonephritis as judged by a marked change in 24-hour sodium or water excretion, an elevation of plasma creatinine concentration or the appearance of bloody urine.

Occasionally, blood would get into the urine and give an elevated urinary sodium concentration. To avoid using urine samples with occult blood, I excluded those experiments in which the acute sodium excretion rate exceeded 2.0 $mEq \cdot min^{-1} \cdot 100g\ BW^{-1}$.

Table 7 summarizes the reasons that observations were discarded in these experiments.

Table 7: Reasons for discarding observations.

	Number of observations	Percentage of total observations
Bloody urine	14	5.7%
Plasma creatinine more than double the value on day of surgery	21	8.5%
Renal or urinary tract pathology at post-mortem	17	6.9%
Sodium excretion rate over 2 microEq·min^{-1}·100g^{-1}	12	4.9%
Valid observations	182	74.0%
Total observations	246	100.0%

DRUGS

Dosage and route of administration for the several drugs which I used are presented in Table 8.

Pentobarbital

It was sometimes necessary to supplement the initial dose of pentobarbital during the surgery for catheter implantation in order to achieve an appropriately deep level of anesthesia.

Ketamine

While I found that ketamine is not an effective anesthetic when given intraperitoneally, it was very effective when administered intravenously. Ketamine caused a remarkable increase in secretion of saliva but there was never any apparent airway obstruction in the several dozen times that this drug was used.

Potassium Penicillin G

Penicillin was prepared weekly and injected into the thigh at the end of surgery.

Hexamethonium

Published values for the intravenous dose of hexamethonium bromide which would produce ganglionic blockade in the rat vary from 1.0 to 3.0 mg/100g BW (Chen, Portman and Wickel, 1951; Salmoiraghi, McCubbin and Page, 1957; Clark, 1971; Numao, Suga and Iriuchijima, 1975; Iriuchijima, 1976; Judy, Watanabe, Henry et al., 1976). I will later discuss my results which lead me to believe that 2 mg/100g i.v. produced effective ganglionic blockade in my preparation.

Acid-Citrate-Dextrose (ACD)

Heparin could not be used as an anticoagulant for donor blood because it frequently impaired hemostasis in the recipient rat. Therefore,

I used ACD as an anticoagulant for donor blood before it was infused into the experimental rat.

PVP-Heparin

It was sometimes possible to maintain patency of vascular catheters by keeping them filled with ordinary saline. The addition of a high molecular weight polymer, polyvinylpyrrolidone (PVP) (360,000 m.w., Nutritional Biochemical Corp., Cleveland, OH) and heparin added to the average duration of catheter patency.

Heparin is known to suppress the rate of aldosterone secretion (Schlatmann, Jansen, Prenen et al., 1964). In order to minimize this effect, I never used solutions in which the heparin concentration exceeded 20 U/ml.

Table 8: Summary of the use of drugs in these experiments.

Drug	Concentration of solution	Dose	Route	Use	Source	Reference
Pentobarbital sodium*	10 mg/ml	3.0-4.5 mg/100g	i.p.	Surgical anesthesia	Pentosol^{R} Burns-Biotec	Barnes and Eltherington 1964
Ketamine HCl*	1 mg/ml	0.5 mg/100g	i.v.	Short-acting anesthetic	Ketalar^{R} Parke-Davis	
Potassium Penicillin G*	250,000U/ml	25,000U	i.m.	Post-surgical anti-microbial	Pfizerpen^{R} Pfizer Labs.	
Hexamethonium Bromide*	10 mg/ml	2 mg/100g**	i.v.	Ganglionic blockade	Sigma Chemical	Judy,Watanabe, Henry et al. 1976
Dimethylphenyl-piperazinium (DMPP)*	50 microg/ml	5 microg/100g	i.v.	Ganglionic stimulator		Bennet and Whitney 1966
Acid-Citrate Dextrose (ACD)***		15 ml/100ml blood		Anticoagulant for donor blood		Frankel,Reitmann & Sonnenwirth 1970
Polyvinyl-pyrrolidone-heparin-saline*	heparin-20 U/ml			Fill dead space of vascular catheters	Nutr.Biochem. Panheprin^{R} Abbot Labs.	

* Prepared in sterile 0.9% saline
** Supplemented with 0.5 mg/100g every 2 minutes during urine collection periods.
*** Prepared in sterile water: trisodium citrate 2.2g/100ml; citric acid 0.8g/100ml; dextrose 2.45g/100ml.

ASSAYS

Creatinine Concentration

Plasma creatinine concentration and creatinine clearance were used to monitor renal function after surgery. Urine samples were 100 microliter aliquots of 24 hour urine collections. One ml venous blood samples collected in heparin at surgery and every 4 to 5 days thereafter provided 200 microliter plasma samples. Plasma and urine creatinine concentrations were determined by a modification of the method of Folin and Wu (Haugen, 1953) using a spectrophotometer (Spectronic 20, Model 2943, Bausch and Lomb):

Standards:

1. Make up 25 ml standards in 0.001N HCl with the following creatinine concentrations: 0, 5, 10, 25, 50, 100, 150, 200 and 300 mg/100ml water. Add 2 drops toluene as preservative and refrigerate.
2. Treat the standards as urine samples and calculate the slope of the least-squares regression line going through the origin that relates optical density to concentration (mg/100ml).

Stock Reagent (Jaffe):

	Urine			Plasma		
2.5N NaOH	3.15 ml	4.2 ml	5.25 ml	0.59 ml	1.50 ml	2.21 ml
Saturated Picric Acid (Sigma)	21.0	28.0	35.0	3.8	9.5	14.25 ml
Water to make	150	200	250	20	50	75 ml

Urine:

1. Add 7.1 ml of urine stock solution to 0.1 ml of urine.
2. Mix in a colorimetric tube and let stand for at least 10 minutes at room temperature.
3. Measure transmittance at 520 millimicrons, calculate optical density and convert to creatinine concentration using the standard curve.

Plasma:

1. To 0.2 ml of plasma (stored at -20C), add the following in sequence to a centrifuge tube:
   0.2 ml distilled water
   0.2 ml 5% (wgt./wgt.) sodium tungstate
   0.2 ml 0.66 Normal H_2SO_4 (7 ml concentrated H_2SO_4 to make 200 ml)
2. Centrifuge to pack the precipitate, decant and save the supernate.
3. Add 2.4 ml of plasma stock solution to the supernate.
4. Mix in a colorimetric tube and let stand for 10 minutes (exactly) at room temperature.
5. Measure transmittance at 520 millimicrons, calculate optical density and convert to creatinine concentration using the standard curve. Use 2.4 ml urine stock solution to 0.8 ml water as a blank. Divide the measured concentration by 4.5 to get the corrected plasma creatinine concentration.

The optical density of the solutions used for urine creatinine concentrations allowed me to measure accurately the urine creatinine concentration. Plasma creatinine concentration determinations, however,

were subject to large errors since the volume of plasma available for analysis was rather small and the optical density was too low for accurate determination of plasma creatinine concentrations. I did not measure the inter-assay variances for plasma creatinine concentration but I did measure significant increases in plasma creatinine concentration one and two days after bilateral nephrectomy. Further, with aliquots of the same plasma sample, my measurements agreed well with the creatinine concentration measurements with an autoanalyzer method.

Sodium and Potassium Concentration

I determined sodium and potassium concentration in urine samples both from 24-hour collections and from 10 minute collections obtained during acute experiments. One 10 microliter aliquot of the 24-hour urine collection was diluted in 5 ml of water which contained 200 ppm of lithium ion (1:500). Three 100 microliter aliquots of each of the 10 minute urine collections were also diluted in 5 ml of 200 ppm lithium (1:50).

To make dilutions, I used disposable 10 microliter (Clay Adams, Parsippany, NJ) and 20 or 100 microliter (Dade Diagnostic Inc., Miami, FL) pipettes without flushing them with diluent. I found that, used in this manner, the pipettes delivered a volume that was different from that indicated on the package. True volumes, determined by weighing the water delivered from 10 pipettes of each size and correcting for the density of water, were 8.7 $\pm$ 0.1 (SEM), 18.6 $\pm$ 0.2 and 95.0 $\pm$ 0.6 microliters for 10, 20 and 100 microliter pipettes, respectively. The true volumes were used to calculate $[Na^+]$ and $[K^+]$.

The diluted samples were stored in rubber-stoppered Pyrex^R test tubes for up to 2 weeks at 4C. Leaching of sodium should not have been a problem with this type of glass. I measured the increase in $[Na^+]$ in a typical dilution stored for periods up to 7 months and estimate that leaching from the glass increased $[Na^+]$ by 2 to 3% per week of storage.

$[Na^+]$ and $[K^+]$ of the dilutions were measured using a flame photometer (Model KY2C, Baird Atomic, Cambridge, MA) with Li^+ as the reference electrolyte. Standard solutions with $[Na^+]$ of 0, 0.05, 0.1, 0.15, 0.2 and 0.3 mEq/l and $[K^+]$ of 0, 0.016, 0.032, 0.048, 0.064 and 0.096 mEq/l

were prepared from sodium and potassium solutions provided by the manufacturer. Lithium solutions were also provided by the manufacturer. All solutions used for flame photometry were prepared with 200 ppm $[Li^+]$.

Quadratic least-squares equations were fit to the relation between the concentrations of standard solutions and the meter reading on the flame photometer. These equations were used to calculate $[Na^+]$ and $[K^+]$ of unknown samples. Standard curves were determined every 25 samples and the concentration of a given sample was obtained by interpolation between the two standard equations determined before and after the measurement of the unknown sample. If the $[Na^+]$ or $[K^+]$ of one of the three aliquots for a given 10 minute urine collection period was clearly different from the other two, I ignored that value in further calculations. For 5% of all observations, the difference between the highest and the lowest values for $[Na^+]$ was more than 85% of the mean $[Na]^+$. These observations were discarded.

Plasma Renin Activity (PRA)

Plasma renin activity was determined from 500 microliter venous blood samples which I drew from conscious, unrestrained rats. I drew the blood into syringes which contained 2 microliters of 300 mM EDTA. The plasma was separated by centrifugation in the syringe at room temperature. 200 microliter samples of plasma were stored at -20C until they were assayed at room temperature. PRA assays were generously provided by Research Services, Veterans' Administration Medical Center, Portland, Oregon. They used a radioimmunoassay method described by Cohen, Grim, Conn et al. (1971). Using this method, Bagby[1] has found intra-assay and inter-assay coefficients of variation of 11% and 16%.

[1] Bagby, Susan P., personal communication.

RENAL HISTOLOGY

I examined the kidneys of some of the rats microscopically. The kidneys were chosen from rats of different ages which had not been surgically-modified and also from experimental rats with a variety of postmortem conditions. I removed the kidneys from rats which were anesthetized with pentobarbital. Immediately after removal, the kidneys were quartered and placed in 10% phosphate-buffered formalin (Frankel, Reitmann and Sonnenwirth, 1970). All tissue was stored for at least a month in the formalin.

The renal tissue was mounted, stained and examined by the Pathology Service, Oregon Regional Primate Research Center, Beaverton, Oregon under the direction of Hideo Uno, M.D. It was imbedded in JB-4 plastic (Polysciences Inc., Warrington, PA) and stained with Gill's Hematoxylin and eosin stain.

Kidney weight and body weight were measured in a series of unmodified SHR and WKY rats which were taken as representative of all the ages used in experimental procedures.

ANALYSIS OF DATA

I analyzed the data according to statistical methods described by Snedecor and Cochran (1967). Differences were considered significant when there was less than a 5% chance of making a type I error.

I did not include paired controls in my experimental design. Consequently, I used an unpaired t-test when testing for differences between two groups.

I had several occasions to use a one-way analysis of variance. For example, I could compare results for the six populations of rats: 2 strains and 3 age groups. In other cases, I wished to study the response to a drug at three or more different doses. In all these cases, I used an analysis that allowed for unequal sample numbers in each group. Because of this, I was particularly careful to check that the data in each group did not strongly deviate from normality and that the variances in each group were similar.

I used a least-squares linear regression equation to describe the renal function curve for each group. In view of the interdependent nature of the two parameters (arterial pressure and sodium excretion rate), I felt justified in using a least-squares analysis that minimized the sum of squares of the horizontal deviations from the regression line.

I used the technique described by Neter and Wasserman (1974) to test for differences between two curves. This test is basically an analysis of variance. It is sensitive to differences both in the slope and position of the two curves. Thus, it is not appropriate by itself to test for differences in the right-left position of two renal function

curves. In order to verify that there was a difference in the right-left positions of the two curves I would first use the analysis of variance to test for differences between the two renal function curves and then test for a difference between the slopes. If there was a difference between two curves that could not be explained as a difference in the slopes, I concluded that one curve was displaced relative to the other curve.

All statistical analyses were performed using programs which I developed for use on the HP-9810 calculator.

RESULTS

CHARACTERISTICS OF THE RATS

Weight and Blood Pressure

Results of the acute experiments were segregated according to the strain and the age of the rats: SHR rats or WKY rats and 8 to 12 weeks, 14 to 19 weeks or 20 to 32 weeks old. Thus, there were six groups in all. I could not perform surgery successfully on rats which were younger than 8 weeks old.

Figure 23 is a summary of body weights of female rats before surgery. Both at 14 to 19 weeks and at 20 to 32 weeks, female WKY rats weighed more than did SHR rats of the same age.

In contrast to the relatively small differences in the weight of SHR and WKY rats are the large differences in mean arterial pressure between SHR and WKY rats which were apparent at all ages. The arterial pressures were measured by direct intraaortic recordings in conscious unrestrained rats from 2 to 4 days after surgery. Figure 24 shows that the mean arterial pressure of both the SHR and the WKY rats had increased significantly with age and that, by analysis of variance, SHR rats were significantly hypertensive relative to age-matched WKY rats.

I found that there was a significant difference between the blood pressures of conscious rats which were used in these experiments and the blood pressures measured after i.v. pentobarbital. Figure 25 depicts a dose-related decrease in mean arterial pressure recorded in SHR and WKY rats from 5 to 6 minutes after pentobarbital infusion. Consequently, the conscious state of the rats in these experiments is an important difference from many previous reports of studies with SHR rats.

Figure 23: Body weight of female SHR and WKY rats. Values are expressed as the mean ± SEM. (n) = number of rats. Differences between group weights were tested by an analysis of variance.

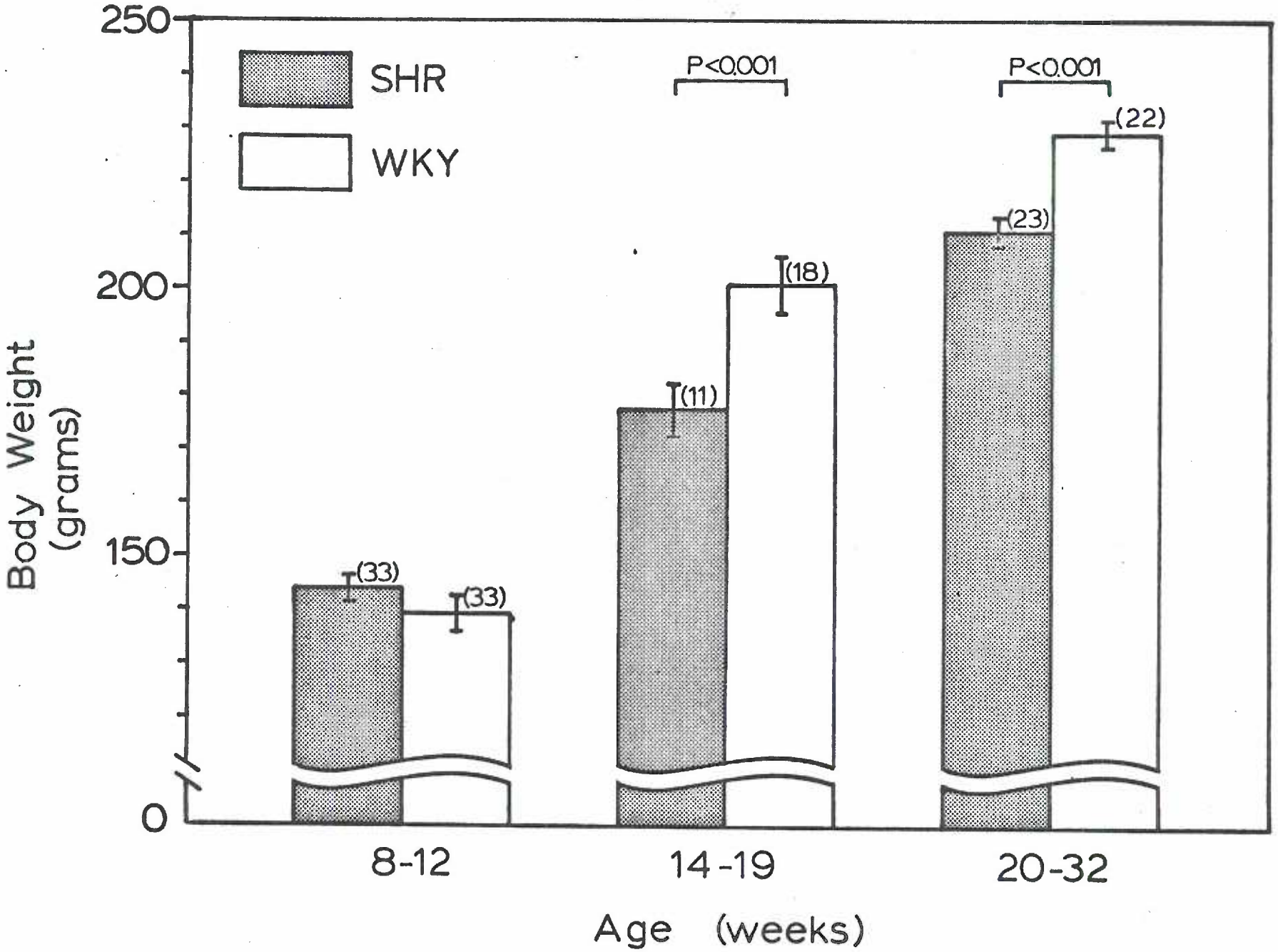

250
200
150
0
SHR
WKY
P<0.001
P<0.001
(33)
(33)
(11)
(18)
(23)
(22)
Body Weight (grams)
8-12
14-19
20-32
Age (weeks)

Figure 24: Mean arterial blood pressure (mmHg) measured in conscious unrestrained rats at least two days after implantation of catheters. Pressures were measured with an aortic catheter. Values are expressed as the mean $\pm$ SEM. (n) = number of rats. Differences between groups were tested by an analysis of variance. Mean arterial pressure for SHR rats was greater than that for WKY rats for each age group ($P < 0.001$). Mean arterial pressures for the oldest group were greater than the pressures for the youngest group for SHR and WKY rats ($P < 0.001$).

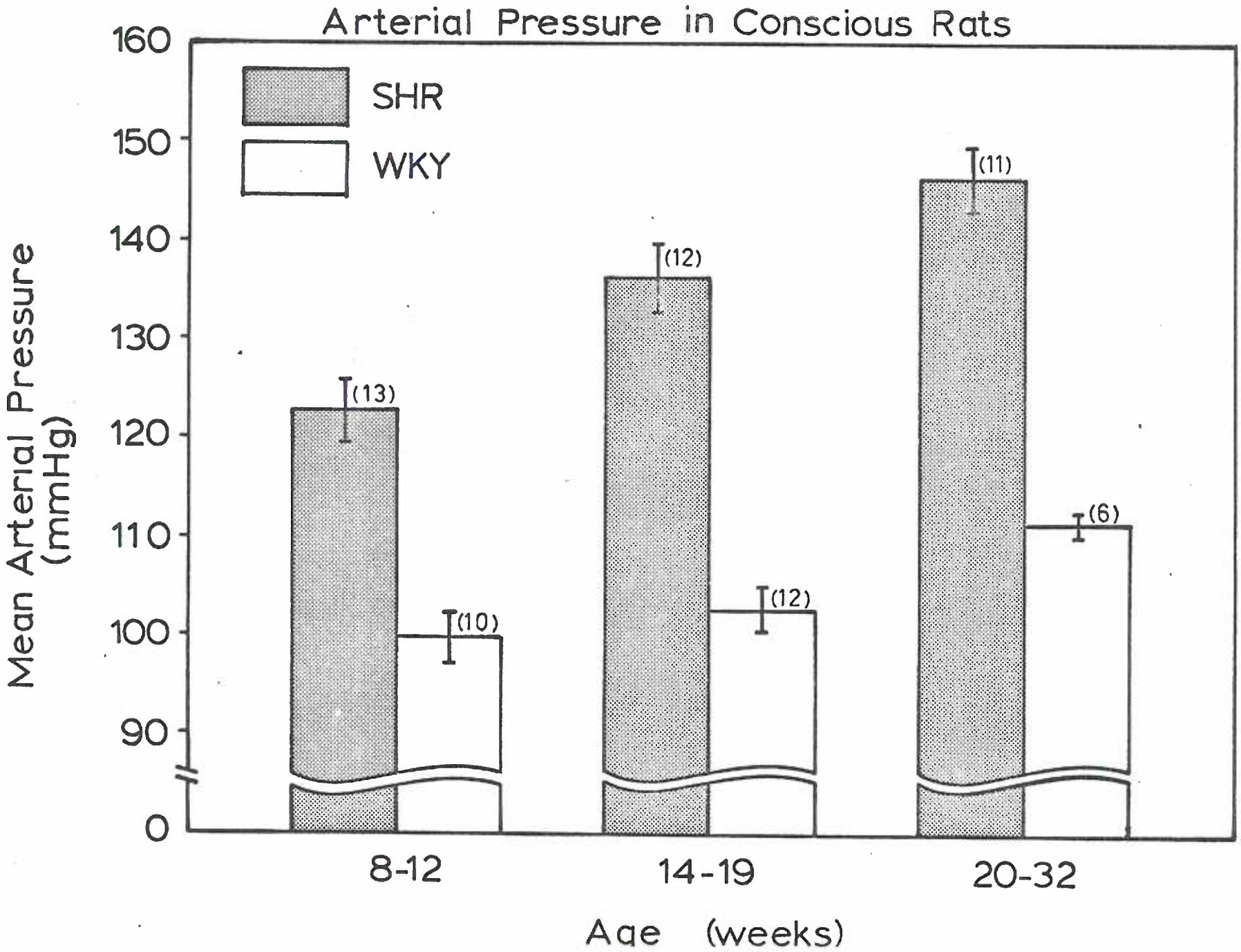
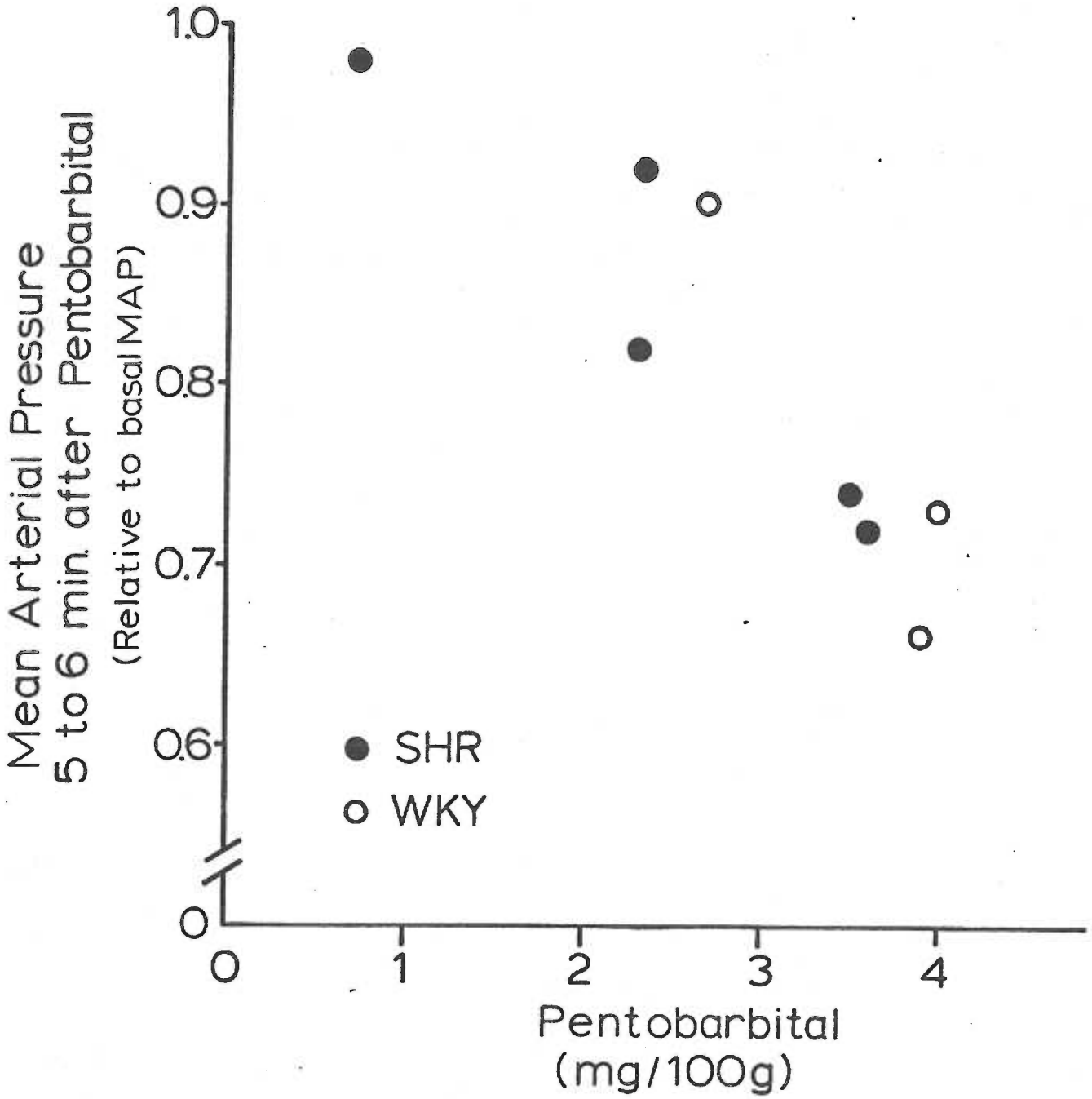

Arterial Pressure in Conscious Rats
SHR
WKY
Mean Arterial Pressure (mmHg)
160
150
140
130
120
110
100
90
0
(13)
(10)
(12)
(12)
(11)
(6)
8-12
14-19
20-32
Age (weeks)

Figure 25: Mean arterial pressure from 5 to 6 minutes after i.v. pentobarbital relative to basal blood pressure. All rats were over 25 weeks old.


Mean Arterial Pressure
5 to 6 min. after Pentobarbital
(Relative to basal MAP)
1.0
0.9
0.8
0.7
0.6
0
SHR
WKY
0
1
2
3
4
Pentobarbital
(mg/100g)

Renal Histology in the Absence of Surgery

There was no evidence of significant pathology in kidneys from either SHR or WKY rats which had no catheters implanted in them. Figures 26 and 27 show that both glomeruli and arteries are essentially normal in SHR and WKY kidneys from rats 18 and 25 weeks old.

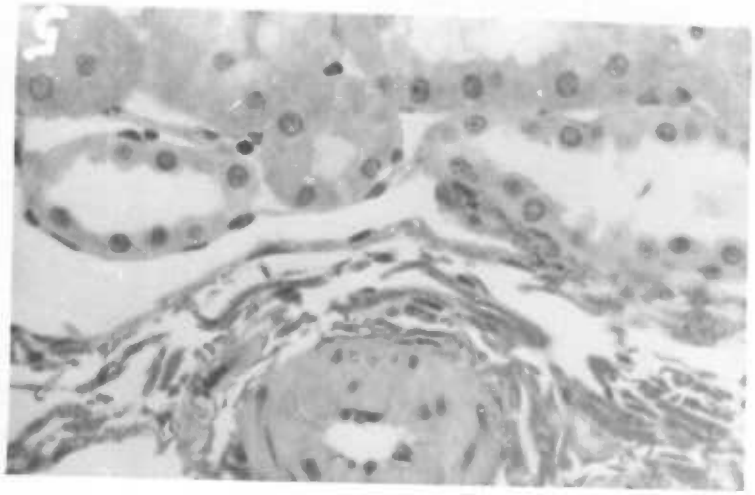
Freis (Freis, Ragan, Pillsbury et al., 1972; Freis and Ragan, 1975) found gross granularity and pitting in kidneys of SHR rats which were more than one year old. Histologic renal pathology which he saw in the kidneys of older SHR rats included nephrosclerosis, fibrinoid necrosis, thickened Bowman's capsule and dilated tubules. None of these pathological features was present in either SHR or WKY kidneys in the absence of surgery.

Figure 26: Photomicrographs of renal tissue from rats without surgery.

Photographs 12 and 13: WKY rat number 78-130 (17.6 weeks old)

Photographs 4 and 5: SHR rat number 78-126 (18.3 weeks old).

Bars on photographs are 100 microns.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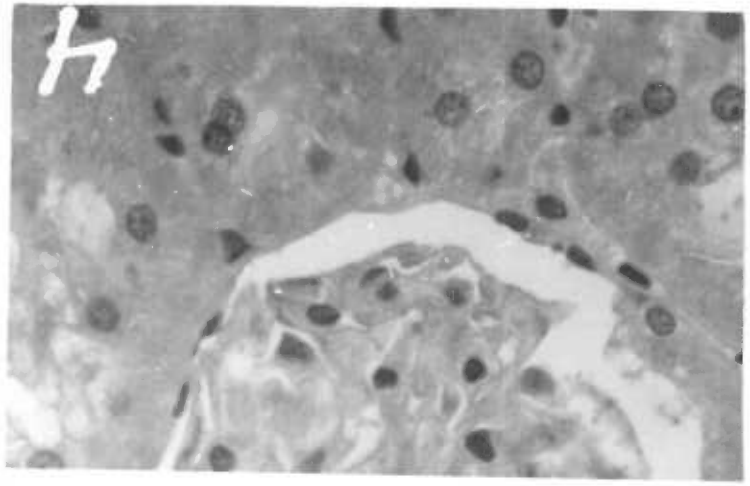
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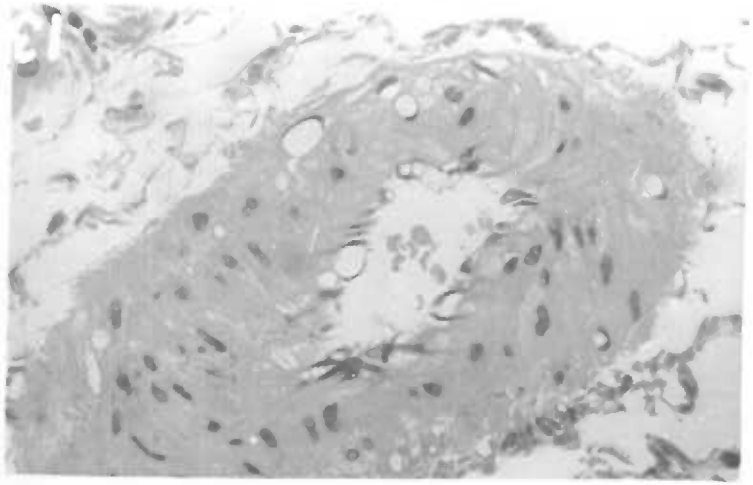


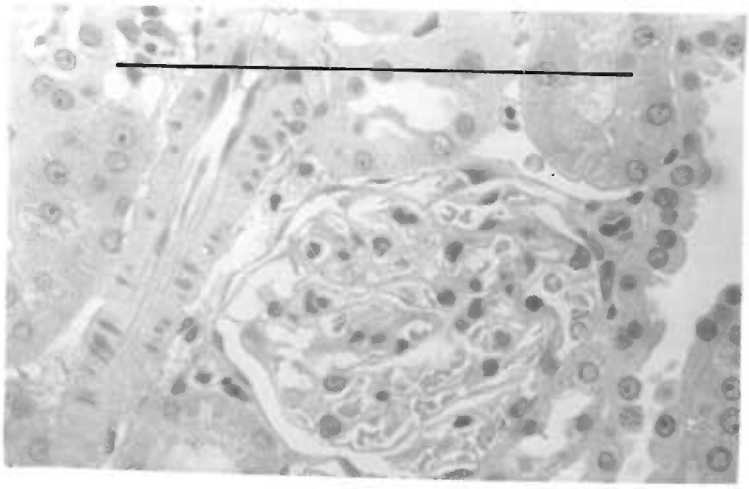
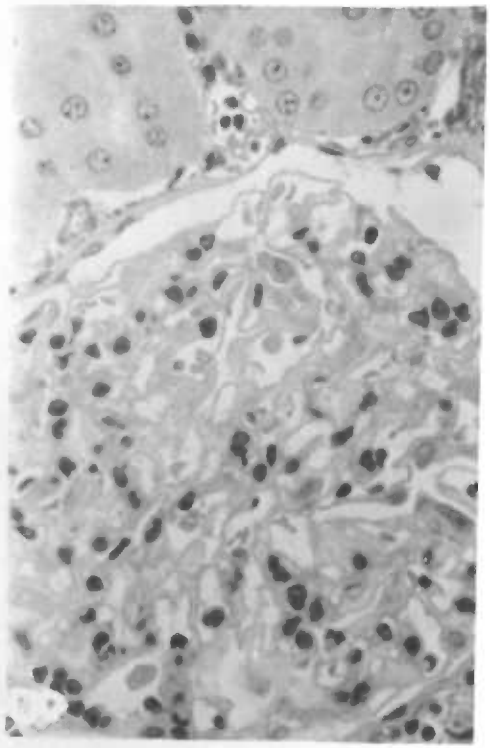


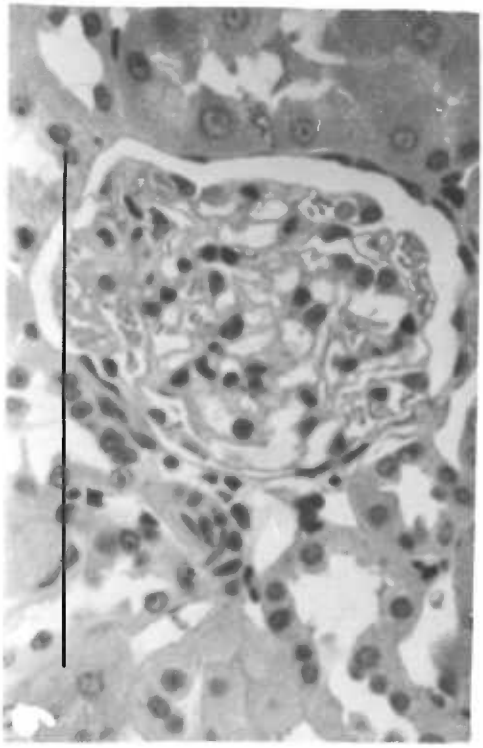
Figure 27: Photomicrographs of renal tissue from rats without surgery.

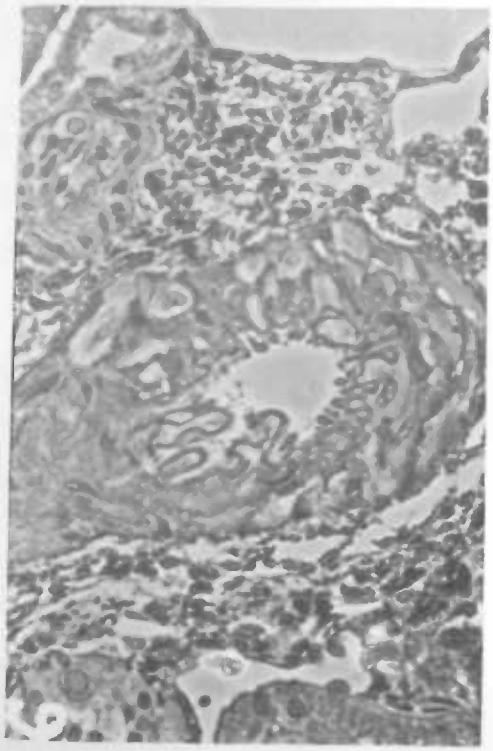
Photographs 6 and 7: WKY rat number 78-127 (24.6 weeks old)

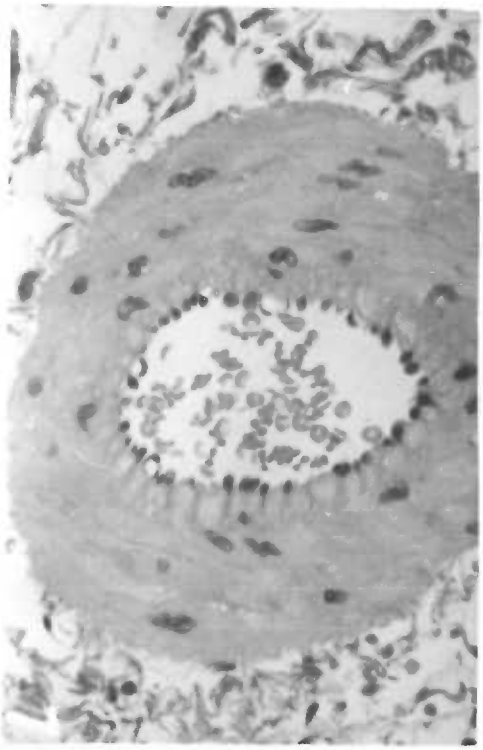
Photographs 8 and 9: SHR rat number 78-128 (24.6 weeks old). Photograph 9 was taken with phase contrast.

Bars on photographs are 100 microns.









VALIDATION OF TECHNIQUES

Bladder Flushing

Using 1/4% acetic acid, I flushed the bladder with 3.5 ml before and with 2.5 ml after the urine collection period. I used the larger volume to flush the bladder before the collection period because the concentration of sodium in the bladder was higher before the collection period than after the period (200 vs. 40 milliEq/liter).

For a bladder diameter of 7 mm, a catheter lumen diameter of 0.7 mm and catheter length of 67 cm, I calculate that the total dead space of the bladder and catheter combined was 0.44 ml. Thus, assuming that the bladder and catheter can be treated as a one-compartment system, I calculate that flushing with 2.5 ml or of 3.5 ml will remove 99.67% or 99.97% of the sodium that was in the bladder before the flush. This agrees with preliminary findings that almost all of the sodium came out of the bladder with the first 2 ml of a flush.

Plasma Renin Activity

--Effect of hexamethonium--

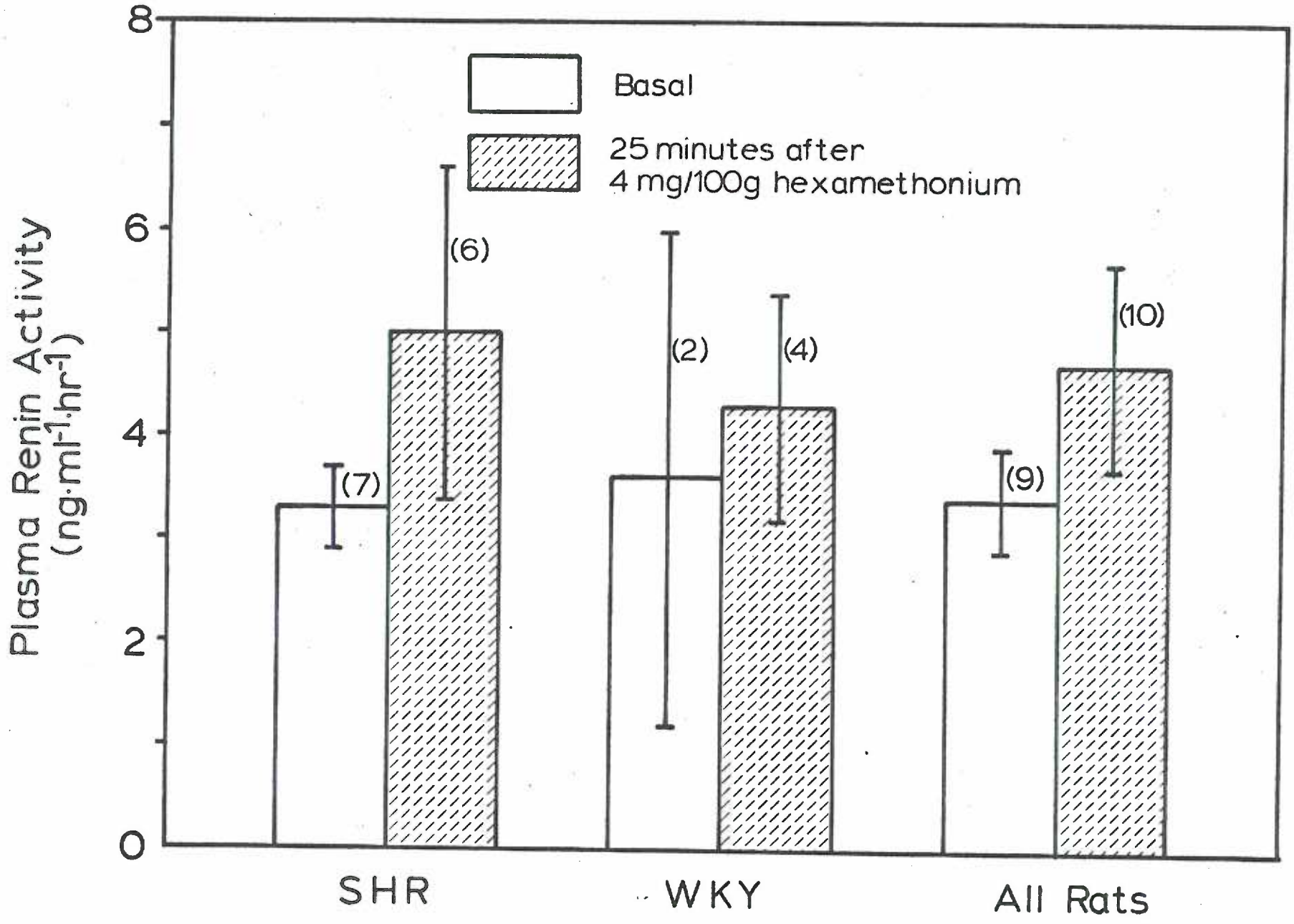
While it was not the objective of this study to examine the role of the renin-angiotensin system in spontaneous hypertension, knowledge of the renin status of the SHR rat will help in the interpretation of the results. I drew blood samples to measure plasma renin activity (PRA) under basal conditions and 25 minutes after infusion of 4 mg/100g hexamethonium i.v. This simulated the conditions that prevailed during acute experiments. The data were analyzed for differences between SHR and WKY rats and between basal condition and after hexamethonium infusion. Figure 28 summarizes these data. By analysis of variance, there were no differences in PRA between the groups.

--PRA of blood from donor rats--

Was there any change in PRA of the experimental rats due to the infusion of hyporeninemic or hyperreninemic blood from a donor rat? I analyzed the results of PRA determinations for the first, second and third milliliters which were drawn continuously at the rate of 1 ml/min. Three ml represents about one-fifth of the total blood volume in these rats. In five rats, PRA of the second and third milliliters which were withdrawn was 130% + 10% (SEM) and 180% $\pm$ 20%, respectively, of the PRA measured with the first milliliter.

Although there was a trend for rising PRA with volume withdrawn, it was not significant. By limiting the amount of blood taken from a donor rat to 2 ml, I believe that I have avoided producing hyperreninemia in the experimental rat.

Figure 28: Plasma renin activity for SHR and WKY rats under resting basal conditions and 25 minutes after 4 mg/100g hexamethonium i.v. Data from 5 SHR rats (age range 10.3 to 25.4 weeks) and 5 WKY rats (age range 9.4 to 21.3 weeks). Mean ± SEM (n) = number of observations.


Basal
25 minutes after
4 mg/100g hexamethonium
Plasma Renin Activity
(ng·ml-1·hr-1)
8
6
4
2
0
(7)
(6)
(2)
(4)
(9)
(10)
SHR
WKY
All Rats

Effectiveness of Ganglionic Blockade

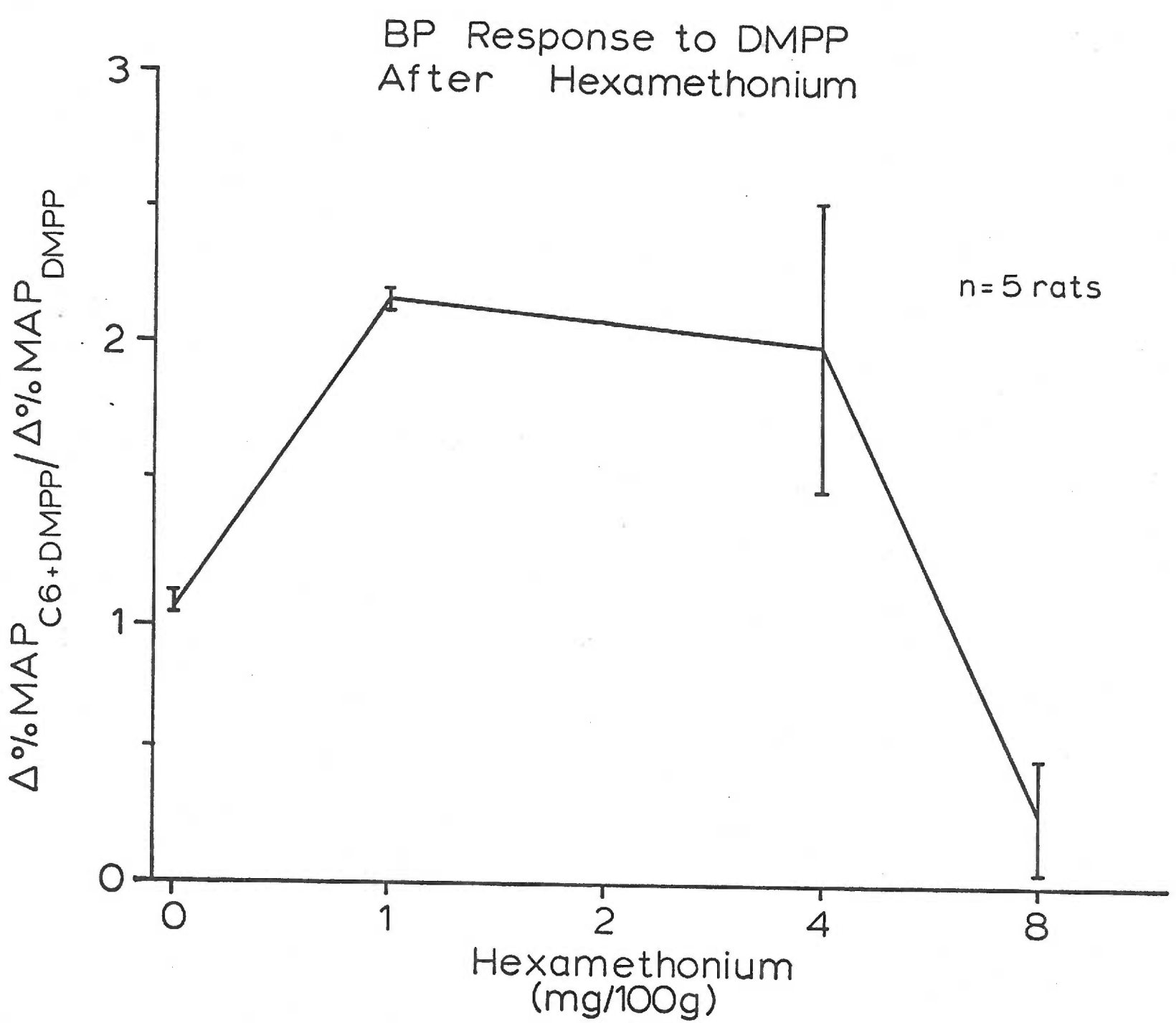
It was important to verify that the dose of hexamethonium which I used to produce ganglionic blockade in the rats did indeed produce the desired blockade. To this end, I observed the difference in response to ganglionic stimulation with and without i.v. hexamethonium.

--Ganglionic stimulation--

I first used the pressor response to 5 microg/100g of dimethylphenylpiperazinium (DMPP) i.v. to provide a measure of ganglionic stimulation. Although 8 mg/100g hexamethonium did block the pressor response to DMPP, this dose of hexamethonium was also lethal in about half the rats. Pre-treatment of rats with 1 or 4 mg/100g hexamethonium actually augmented the pressor response to subsequent doses of DMPP (Figure 29). DMPP may act directly to release post-ganglionic neurotransmitters (Bennet and Whitney, 1966; Brus and Jacobowitz, 1970) and it is possible that hexamethonium produces a denervation hypersensitivity (Cannon, 1939) that is expressed with DMPP stimulation.

Therefore, I rejected DMPP for the assessment of ganglionic blockade and, instead, used the change in pulse rate after vagal stimulation. In male SHR and WKY rats which were anesthetized with 3 to 4 mg/100g pentobarbital i.p., I ligated the left vagus nerve and isolated the right vagus nerve. I then ligated the right vagus nerve and placed bipolar stimulating electrodes on the nerve caudal to the ligated section. A stimulator (Model SM9B, Grass Instruments, Quincy, MA) was adjusted so that a 4 second vagal stimulation would produce an 80% decrease in pulse rate. Hexamethonium was then administered into the right external jugular vein in an initial bolus of 2 mg/100g followed by 0.5 mg/

Figure 29: The effect of hexamethonium on the pressor response to 5 microg/100g DMPP. The pressor response to DMPP (Δ $\%MAP_{DMPP}$) is the quotient of the change in mean arterial pressure (MAP) from basal conditions to 30 seconds after infusion of 5 microg/100g DMPP i.v. divided by the MAP after DMPP infusion. The relative response to DMPP is the ratio of the response to DMPP without hexamethonium and the response to DMPP measured 1 minute after i.v. hexamethonium (Δ $\%MAP_{C-6 + DMPP}$). Mean $\pm$ SEM for 5 rats. Analysis of variance reveals no significant differences ($P > 0.05$) but the responses at 1 and 4 mg/100g were different from the response at 0 mg/100g with $P < 0.01$.


BP Response to DMPP
After Hexamethonium
n=5 rats
Δ%MAP C6+DMPP / Δ%MAP DMPP
3
2
1
0
0
1
2
4
8
Hexamethonium
(mg/100g)

100g 2 and 4 minutes later. Figure 30 shows that hexamethonium completely blocked the negative chronotropic effect of vagal stimulation for at least 20 minutes. Presumably, hexamethonium also blocks renal ganglia (p 196).

--Hypotensive effect of hexamethonium--

Additional evidence that 2 mg/100g i.v. hexamethonium produces ganglionic blockade is the dose-response curve of hypotension which was produced by the drug (Figure 31). All doses of intravenous hexamethonium produced a significant fall in blood pressure by analysis of variance. There was, however, no dose-related hypotension beyond that which was produced by 1 mg/100g. Since hexamethonium-induced vasodilation is mediated by ganglionic blockade (Wien, Mason, Edge et al., 1952), I have taken my data to mean that the hexamethonium dose which I used for experiments (2 mg/100g, i.v.) produced adequate ganglionic blockade.

I found that young SHR and young WKY rats had different hypotensive responses to hexamethonium (Figure 32). The mean arterial pressure of conscious young WKY rats fell by a mean of 38% during the 10 minute period after hexamethonium infusion. The same dose of hexamethonium in the young SHR caused only a 22% fall in mean arterial pressure. This difference in response to hexamethonium was also evident when the data were analyzed in terms of the absolute rather than the percentage change in arterial pressure. See Figure 33 for a depiction of mean arterial pressures before and after hexamethonium infusion.

There were no differences in the pressure response to hexamethonium between the 14 to 19 or 20 to 32 week old groups of rats. The young SHR rats were the only group which was relatively refractory to the hypotensive effects of hexamethonium.

Figure 30: Ratio of the heart rate after 4 seconds of the electrical stimulation of the right vagus nerve for 4 seconds (HR_{STIM}) and the heart rate before stimulation ($HR_{\emptyset}$). Data are from three rats before and after the intravenous administration of hexamethonium bromide (C-6).

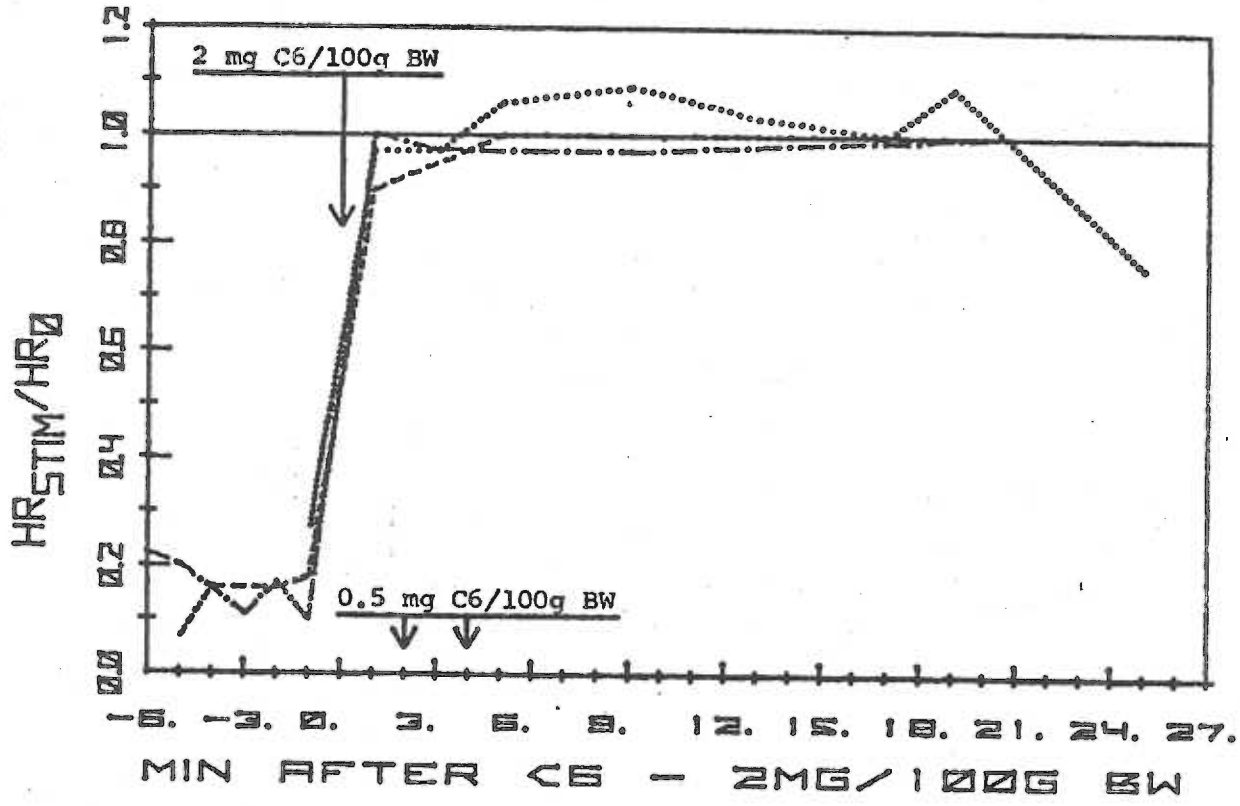

2 mg C6/100g BW
0.5 mg C6/100g BW
HR STIM/HR0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
-6. -3. 0. 3. 6. 9. 12. 15. 18. 21. 24. 27.
MIN AFTER C6 - 2MG/100G BW

Figure 31: Percent change in mean arterial pressure (MAP)30 seconds after i.v. hexamethonium. The data were from 12 rats including SHR rats (4), WKY rats (4) and others (4). Data are presented as the mean ± SEM. (n) = number of observations. Significance tested vs. no fall in mean arterial pressure.

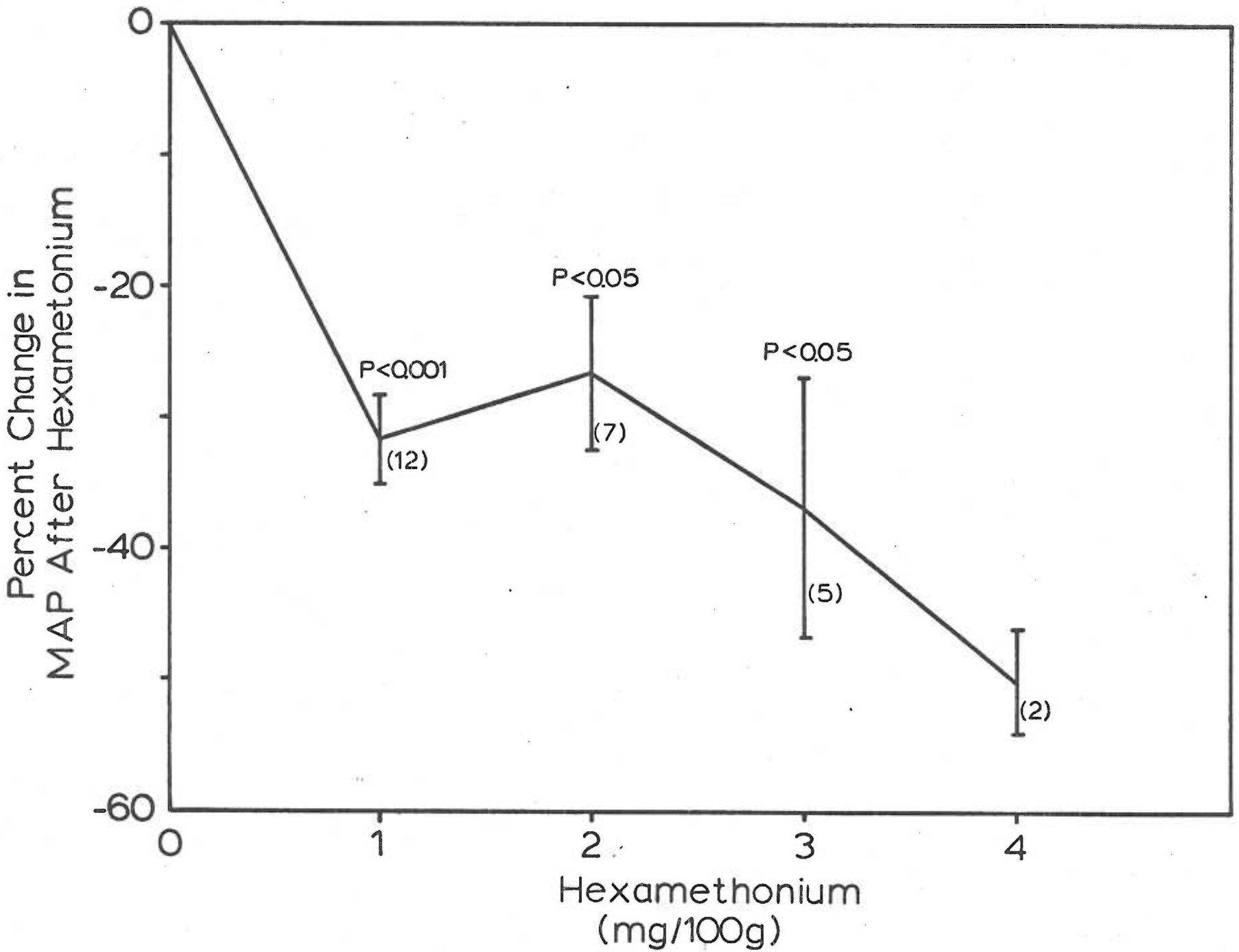

0
-20
-40
-60
Percent Change in
MAP After Hexametonium
P<0.001
P<0.05
P<0.05
(12)
(7)
(5)
(2)
0
1
2
3
4
Hexamethonium
(mg/100g)

Figure 32: Ratio of the mean arterial pressure in the basal state (MAP_{Basal}) and pressure during the 10 minutes after the injection of 2 mg/100g BW hexamethonium i.v. (MAP_{C-6}). Mean $\pm$ SEM (n) = number of observations. Comparisons by analysis of variance.

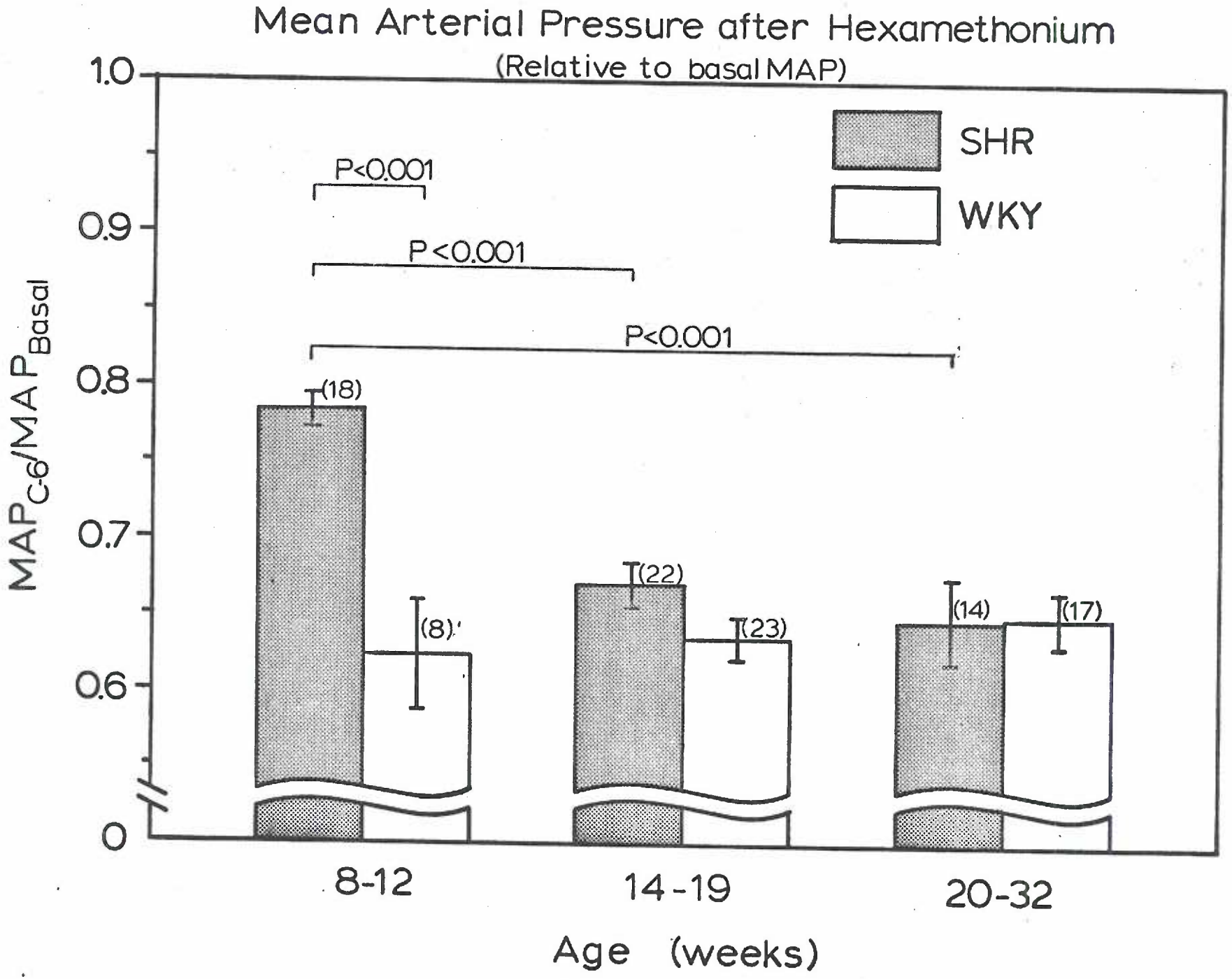
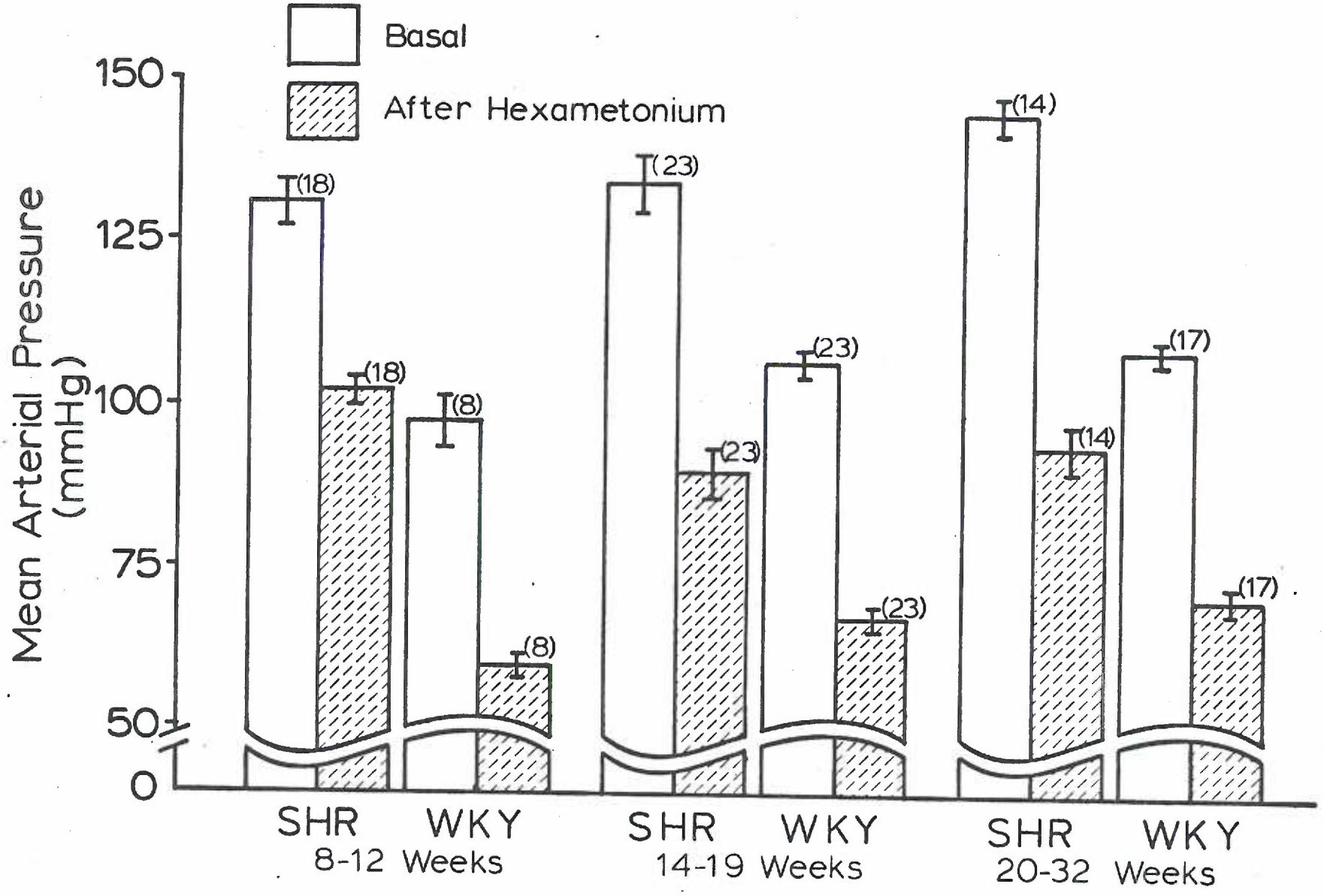

Mean Arterial Pressure after Hexamethonium
(Relative to basal MAP)
1.0
0.9
0.8
0.7
0.6
0
SHR
WKY
P<0.001
P<0.001
P<0.001
(18)
(8)
(22)
(23)
(14)
(17)
8-12
14-19
20-32
Age (weeks)
MAP C-6/MAP Basal

Figure 33: Mean arterial pressure in female rats of various ages and strains before and after the infusion of 2 mg/100g hexamethonium. Arterial pressure after hexamethonium was determined as the arithmetic average of the mean arterial pressure for the first 10 minutes after the infusion. Mean ± SEM. (n) = number of observations.


Basal
After Hexametonium
Mean Arterial Pressure (mmHg)
150
125
100
75
50
0
(18)
(18)
(8)
(8)
(23)
(23)
(23)
(23)
(14)
(14)
(17)
(17)
SHR
WKY
8-12 Weeks
SHR
WKY
14-19 Weeks
SHR
WKY
20-32 Weeks

EFFECTS OF SURGERY

Weight Loss

Rats were weighed before surgery and again at the post-mortem examination. After surgery, rats grew less rapidly than would have been predicted using regression curves of pre-surgery weight and age (Figures 9 through 12, pp 77-80) to estimate the expected weight gain in the absence of surgery.

Figure 34 plots the ratios of actual to predicted weight for male rats with only a venous catheter. Figure 35 presents the same data for female rats which each had an aortic, a venous and a bladder catheter implanted. After surgery, most rats did not grow as rapidly as did the rats without surgery. Most of the relative weight loss occurred within a few days after surgery in those rats with three catheters. Relative weight loss was more gradual in rats with a venous catheter only. SHR and WKY rats both showed the same pattern of weight loss.

The predicted weight was determined from the results of weights from rats which had been maintained in cages with other rats. Rats with catheters were kept in individual cages. It has been shown that isolation of rats is associated with a slower growth rate. Hatch, Wiberg, Zawidzka et al. (1965) found that female Wistar rats raised in individual cages for 13 weeks after weaning weighed 20% less than rats which were raised in community cages; male rats raised in individual cages weighed 9% less than control rats. Thus, some of the weight loss that I observed may be related to the fact that the rats were isolated after surgery.

Figure 34: Ratio of the actual weight to the predicted weight after surgical implantation of a catheter in the right external jugular vein for male SHR and WKY rats. The predicted weight was obtained from the growth curves for rats without surgery (Figures 11 and 12). The actual weights were measured at post-mortem examination.

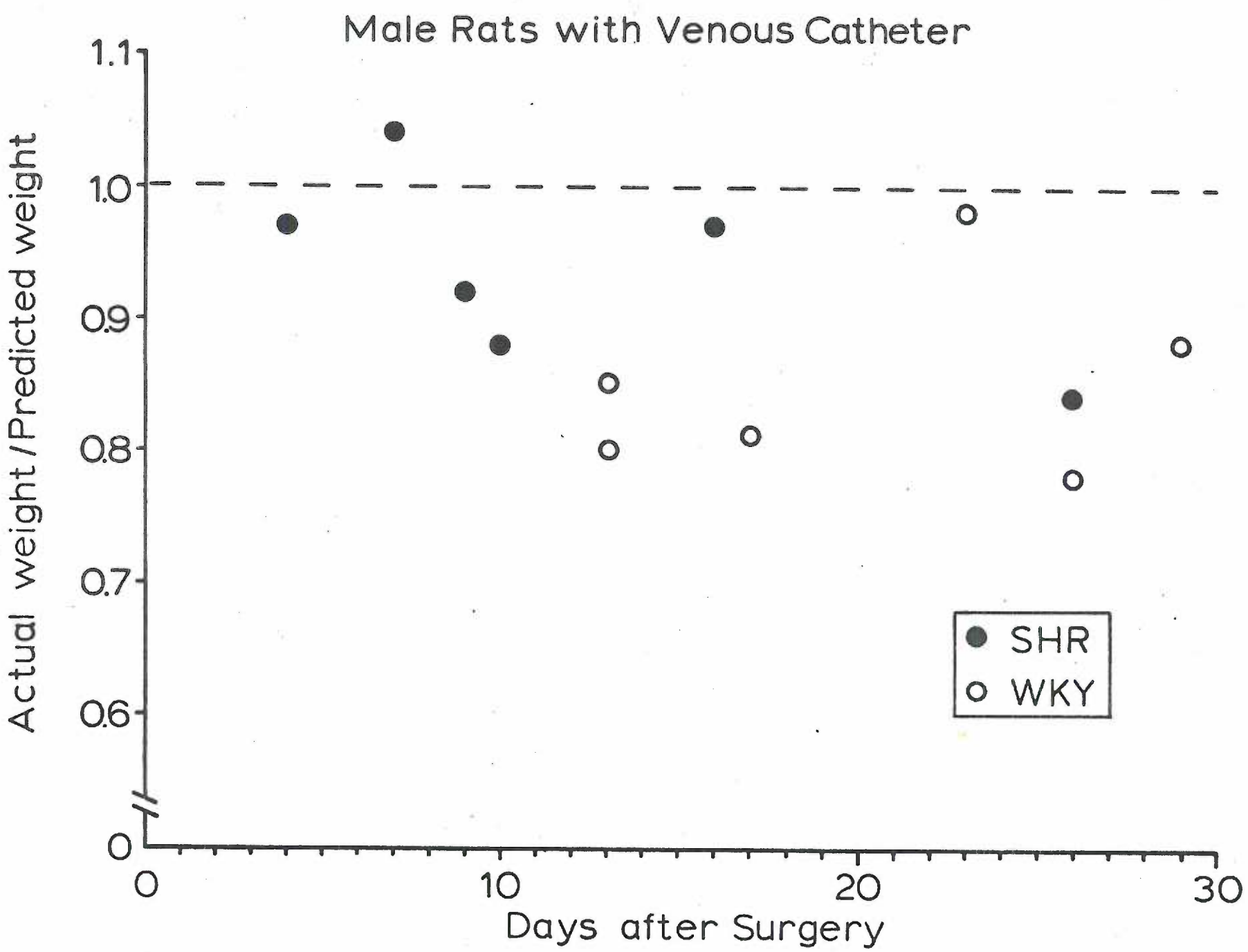
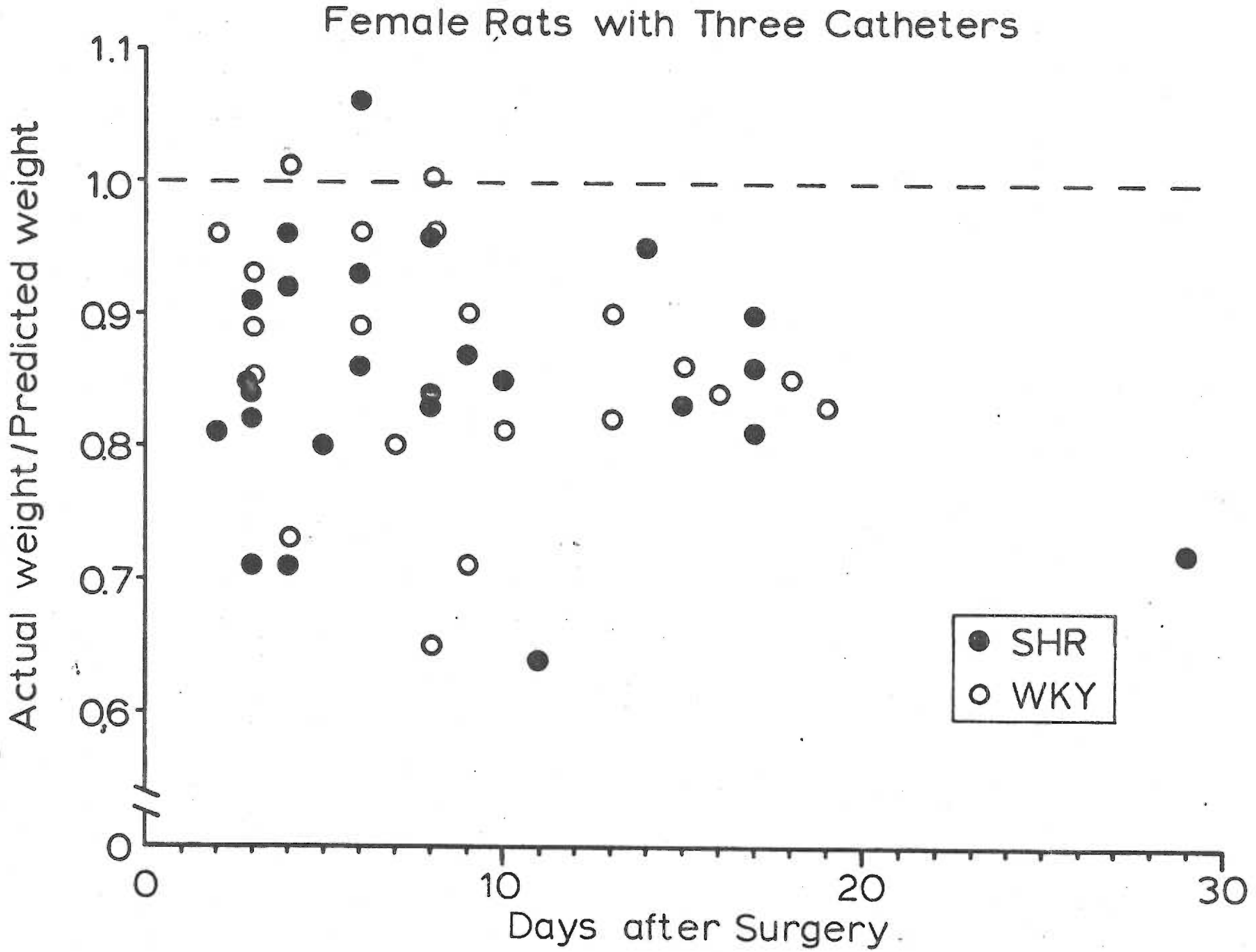

Male Rats with Venous Catheter
Actual weight / Predicted weight
1.1
1.0
0.9
0.8
0.7
0.6
0
0
10
20
30
Days after Surgery
SHR
WKY

Figure 35: Ratio of the actual weight to the predicted weight after the surgical implantation of catheters in the right external jugular vein, abdominal aorta and urinary bladder for female SHR and WKY rats. The predicted weight was obtained from the growth curves for rats without surgery (Figures 9 and 10). The actual weights were measured at post-mortem examination.


Female Rats with Three Catheters
Actual weight/Predicted weight
1.1
1.0
0.9
0.8
0.7
0.6
0
0
10
20
30
Days after Surgery
SHR
WKY

Duration of Catheter Patency

I analyzed my last 100 surgical preparations to ascertain the failure rate of the various catheter types. There is no unbiased estimate of the length of time for which catheters remained functional; many rats had one or more patent catheters when they were killed for reasons other than catheter failure.

In those animals where catheters did fail and the duration of patency was known, the median number of days for which the catheter remained patent was 5, 5 and 3 for the aortic, venous and bladder catheters, respectively. This is a conservative estimate since there were several animals for which all catheters remained functional for a month or more. If a catheter remained open for a few days after surgery, it was likely to remain patent and functional for several weeks.

Chronic Excretion Rates of Water, Sodium and Creatinine

Figures 36, 37 and 38 present data on the excretion rates of volume, sodium and creatinine under chronic conditions. All data were derived from the analysis of daily urine collections from 6 representative conscious rats. The six rats were selected because, in each case, catheters remained functional for at least one week after surgery. The excretion rates are normalized to the pre-surgery body weight of the rat.

While there was a great deal of individual variation, there was a trend for urine flow to increase from about 5 microliters·min^{-1}· 100 g^{-1} for two days immediately after surgery to from 5 to 15 microliters·min^{-1}· $100g^{-1}$ for the next two weeks. Some of the variation in urine flow may be accounted for by fluctuations in the ambient laboratory temperature.

The sodium excretion rate was low for a few days after surgery and increased to about 1 microEq·min^{-1}· $100g^{-1}$ by the third day after surgery. The low sodium excretion rate immediately after surgery was expected since there was often a loss of extracellular fluid during surgery and the rats did not drink for up to 12 hours after surgery.

Some of the rats showed a high rate of creatinine excretion for a day or two after surgery. This could be expected on the basis of surgical trauma. After that, creatinine excretion rate was quite constant for any given rat.

Figure 36: Urine flow rate (microliters·min^{-1}·100g BW^{-1}) for 6 representative rats after the surgical implantation of catheters in the right external jugular vein, abdominal aorta and urinary bladder.

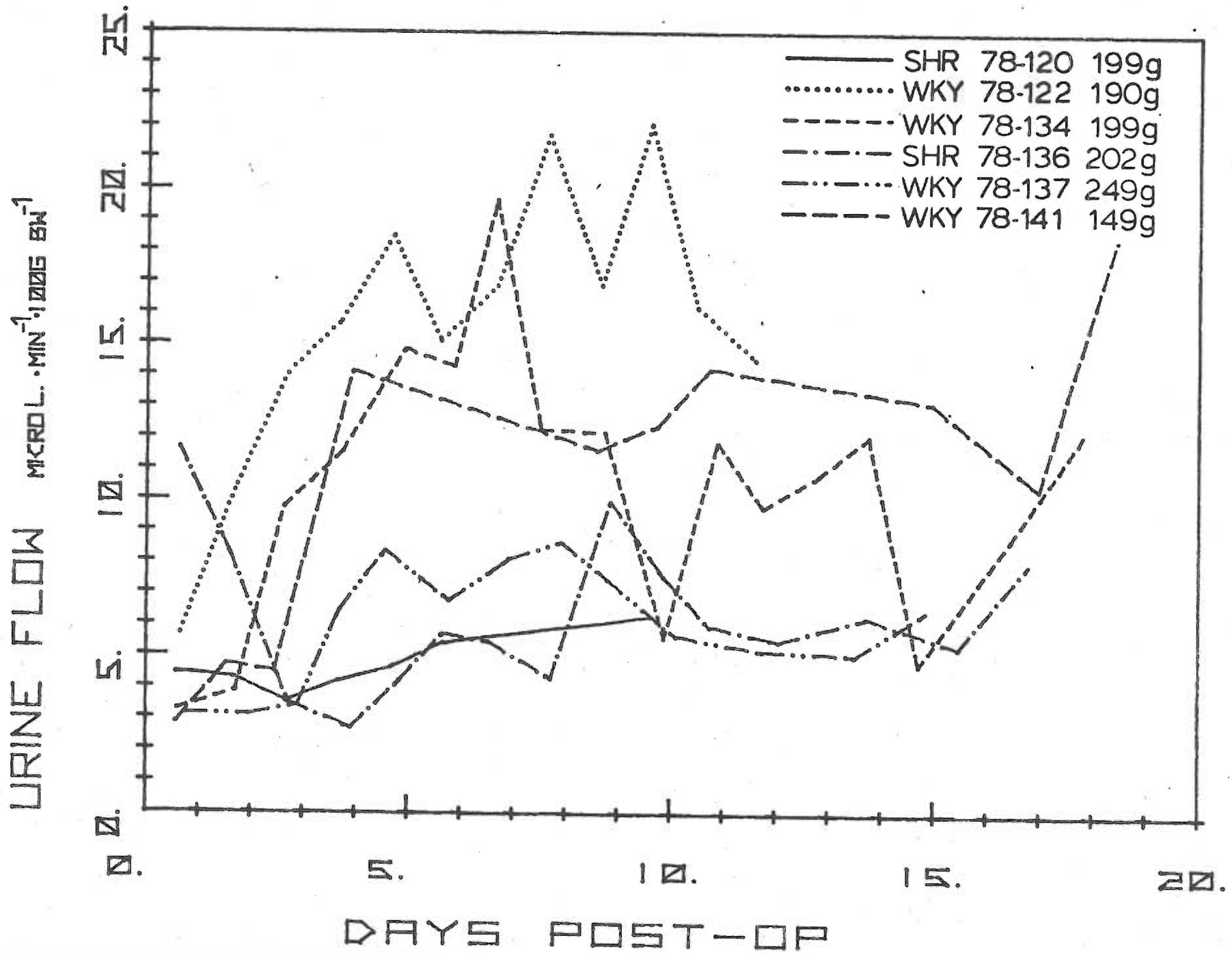

URINE FLOW MICRO L. · MIN^{-1} · 100G BW^{-1}
0.
5.
10.
15.
20.
25.
0.
5.
10.
15.
20.
DAYS POST-OP
SHR 78-120 199g
WKY 78-122 190g
WKY 78-134 199g
SHR 78-136 202g
WKY 78-137 249g
WKY 78-141 149g

Figure 37: Sodium excretion rate (microEq·min^{-1}·100g BW^{-1}) for 6 representative rats after the surgical implantation of catheters in the right external jugular vein, abdominal aorta and urinary bladder.

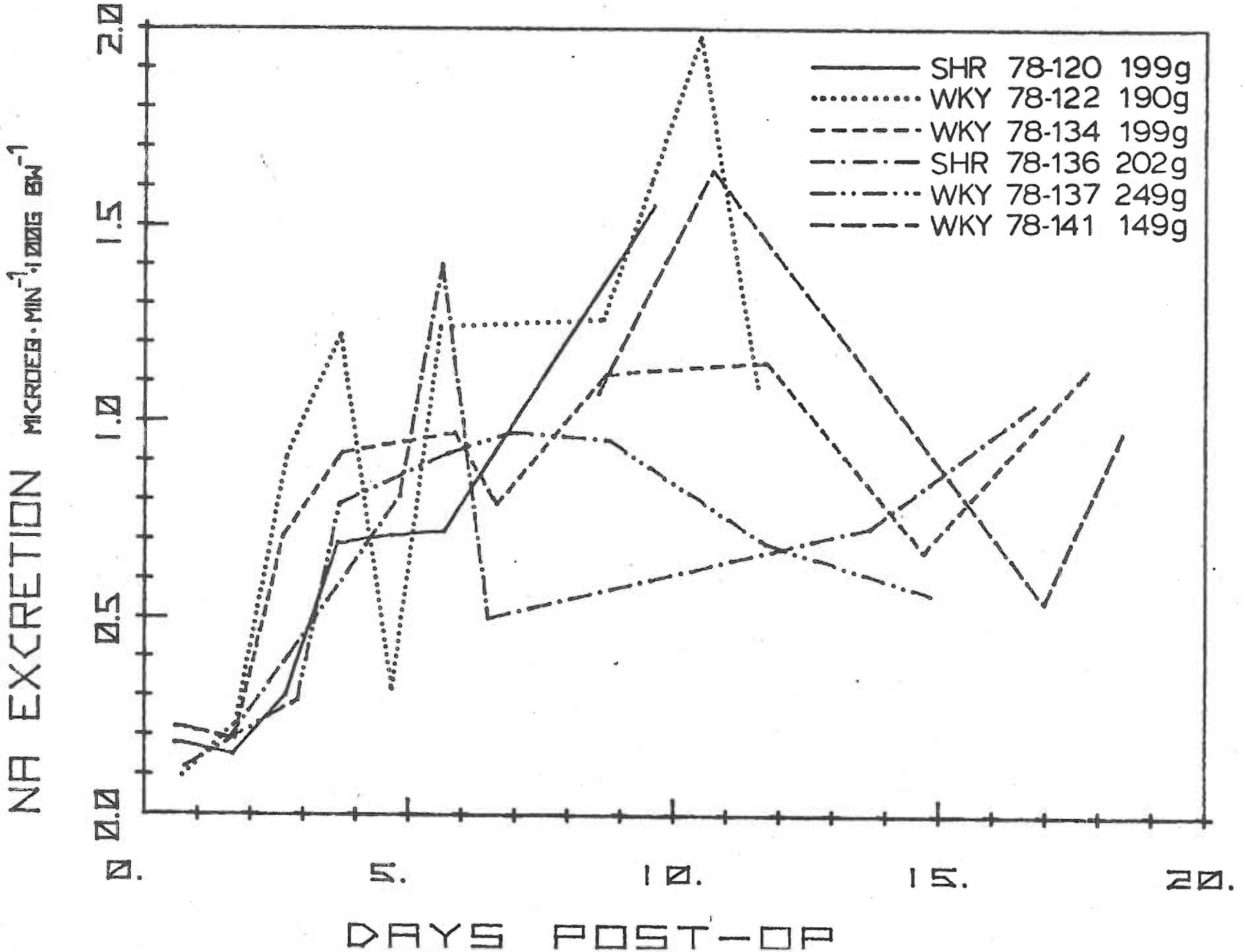
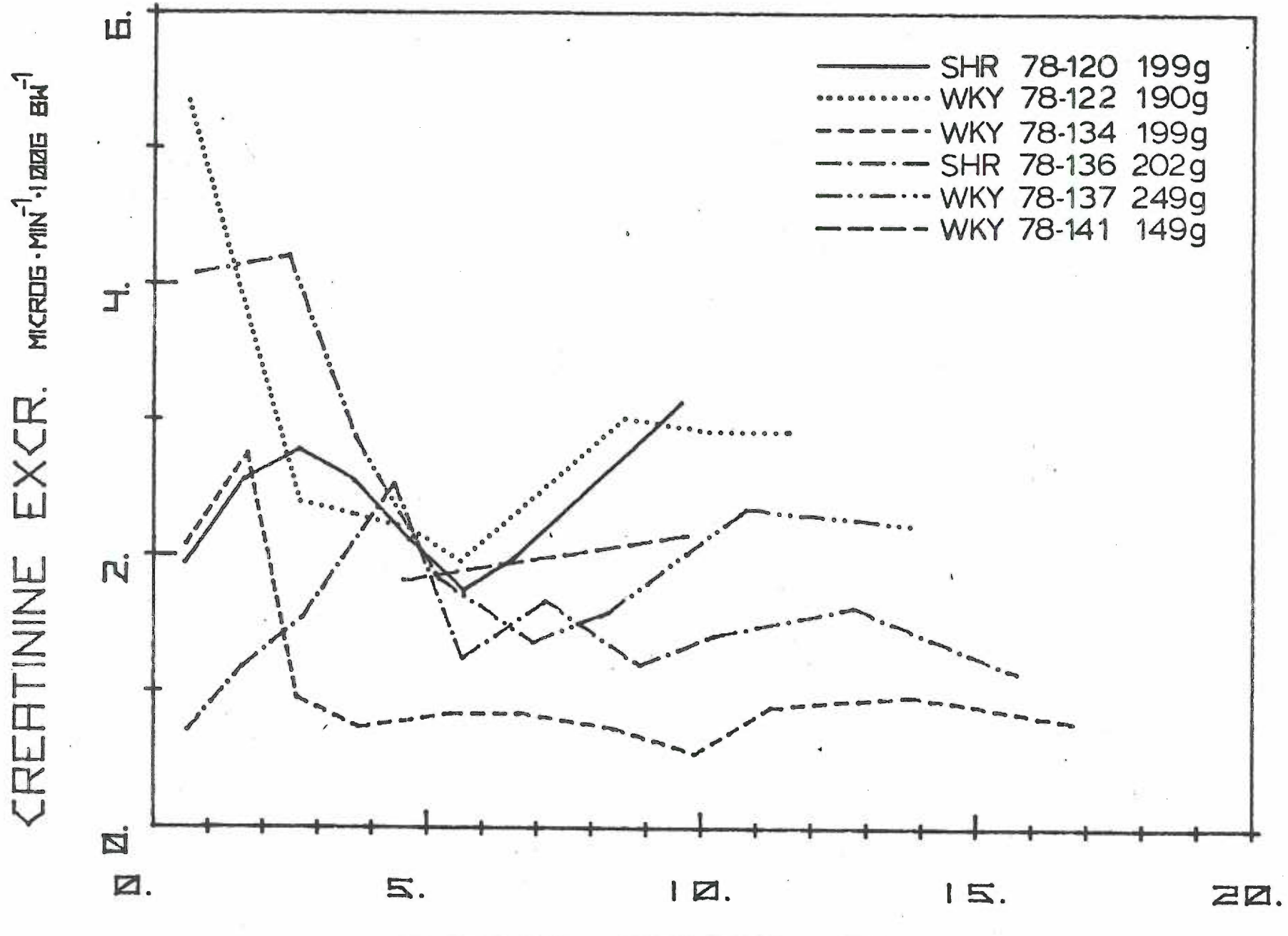

NA EXCRETION MICROEQ·MIN⁻¹·100G BW⁻¹
2.0
1.5
1.0
0.5
0.0
0.
5.
10.
15.
20.
DAYS POST-OP
SHR 78-120 199g
WKY 78-122 190g
WKY 78-134 199g
SHR 78-136 202g
WKY 78-137 249g
WKY 78-141 149g

Figure 38: Creatinine excretion rate (microg·min^{-1}·100g BW^{-1}) for 6 representative rats after the surgical implantation of catheters in the right external jugular vein, abdominal aorta and urinary bladder.


CREATININE EXCR. MICROG·MIN⁻¹·100G BW⁻¹
0.
2.
4.
6.
0.
5.
10.
15.
20.
DAYS POST-OP
SHR 78-120 199g
WKY 78-122 190g
WKY 78-134 199g
SHR 78-136 202g
WKY 78-137 249g
WKY 78-141 149g

Evaluation of Renal Failure

I used three major methods to decide whether a rat had renal failure on a given day of data collection. First, I measured the daily urine volume and inspected the urine for the presence of blood. Second, I assayed periodic plasma samples for creatinine concentration. Thirdly, I examined the urinary tract at post-mortem for the presence of occlusion or of infection. If any of these procedures revealed an abnormality, the data collected from that rat was excluded unless there was reason to believe that some of the data was collected before the development of the renal lesion which was seen post-mortem. I shall describe the results of these observations in some more detail.

Creatinine clearance was normal after surgery. Creatinine excretion rate (Figure 38) was 1.98 $\pm$ 0.25 (SEM) microg$\cdot$min$^{-1}\cdot$100g^{-1} (n=6) after the second day after surgery. Since the plasma creatinine concentration for experimental rats averaged 0.25 $\pm$ 0.03 (SEM) mg/100 ml (n=27), one can calculate that the mean creatinine clearance was 0.79 ml$\cdot$min$^{-1}\cdot$100g^{-1}. The values for plasma creatinine concentration and creatinine clearance are similar to the values reported by Feld, Van Liew, Galaske et al. (1977) for 8 to 43 week old SHR and WKY rats. These workers also agree with my finding that the plasma creatinine concentration and the creatinine clearance of SHR and WKY rats are identical.

If the plasma creatinine concentration had doubled between the day of surgery and the measurement of pressure and sodium excretion rate for a renal function curve, the data were excluded from the delineation of the renal function curves.

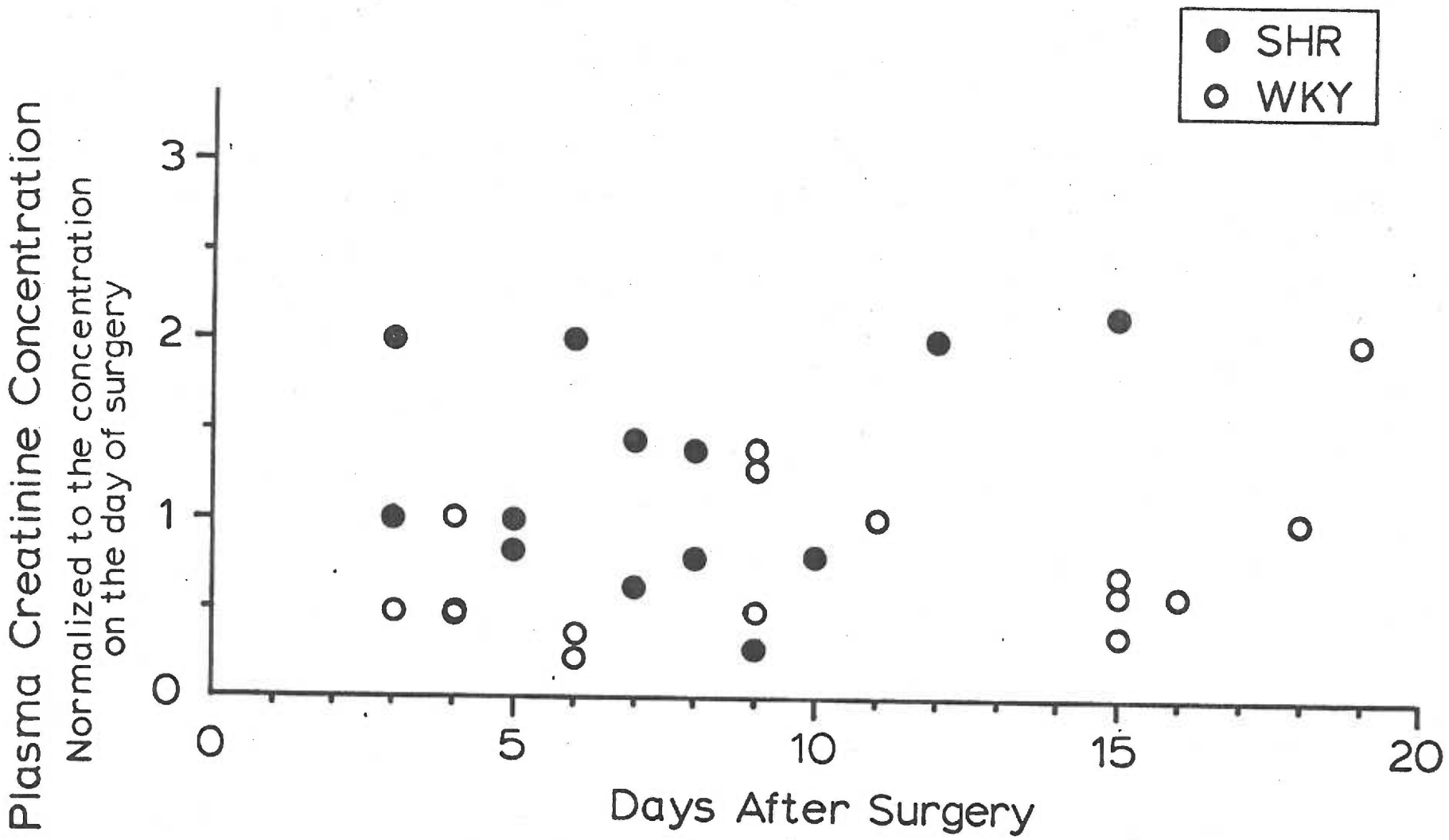
Figure 39 illustrates the plasma creatinine concentrations after surgery for catheter implantation relative to the concentration on the day of surgery for the period during which rats contributed data to renal function curves.

Analytical errors for the 500 microliter blood samples which were available from the rats contributed to the large variance of measured plasma creatinine concentration. The spectrophotometer which I used required samples which were diluted to a volume of at least 3 ml. The concentration of creatinine at this dilution for plasma samples was near the limits of sensitivity of the spectrophotometer.

I used plasma creatinine concentration rather than creatinine clearance as an index of renal failure since increased plasma creatinine concentration was consistently associated with a decrease in creatinine clearance.

Gross examination of the urinary tract of rats with three catheters implanted revealed that in 9% of the rats the bladder was either dilated or that there was a bladder infection. The right kidney was infected or the right ureter was dilated in 24% of the rats; the left kidney or ureter was abnormal in 23% of the rats. Overall, there was gross urinary tract pathology in 39% of the rats which were examined. In every case for which pathology was evident at post-mortem examination, I either discarded the data from the rat or I only used data obtained on an earlier date before there was an apparent change in renal excretion. For these purposes, I used the urine flow or the plasma creatinine concentration to assess the daily renal excretory function.

Figure 39: The ratio of plasma creatinine concentration after surgery to the plasma creatinine concentration on the day of surgery in female SHR and WKY rats with catheters in the external jugular vein, the abdominal aorta and the urinary bladder. All the data were obtained from rats during the time that they were contributing data for renal function curves.


SHR
WKY
Plasma Creatinine Concentration
Normalized to the concentration
on the day of surgery
0
1
2
3
0
5
10
15
20
Days After Surgery

Some degree of renal pathology was evident histologically in those rats which had urinary tract obstruction. In rats numbered 77-45, 77-104 and 77-106, I inserted the bladder catheter by the old method which involved ligation of the neck of the bladder. Rat number 77-45 (Figure 40, Photograph 16) was hydronephrotic and the kidney had a very thin cortex. A section of cortex from rat number 77-104 (Figure 40, Photograph 18) showed tubular degeneration and rat number 77-106 (Figure 40, Photograph 18) had arteriolar hyalinization. For rat number 78-99 I used a new bladder catheterization procedure in which I ligated the urethra distal to the bladder. I used this newer procedure for all rats which were used to define the renal function curves. Rats were much less likely to develop urinary tract obstruction with the new procedure than when I ligated the neck of the bladder. Rat number 78-99, however, had been anuric for 2 days when it was killed. In this rat there were mild tubular changes with vacuolization (Figure 40, Photograph 23).

Figure 40: Photomicrographs of renal tissue from rats with surgical implantation of catheters in the external jugular vein, the abdominal aorta and the urinary bladder.

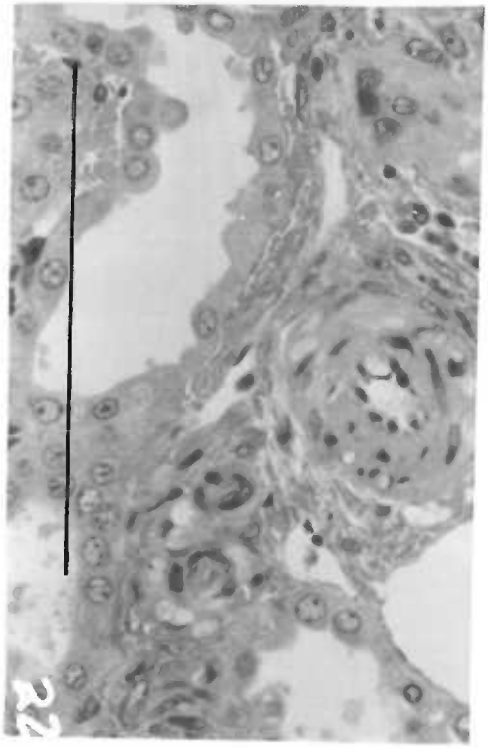
Photograph 16: Wistar rat number 77-45 47 days after surgical implantation of catheters using the old technique for catheterization of the bladder. Thin cortex of hydronephrotic kidney.

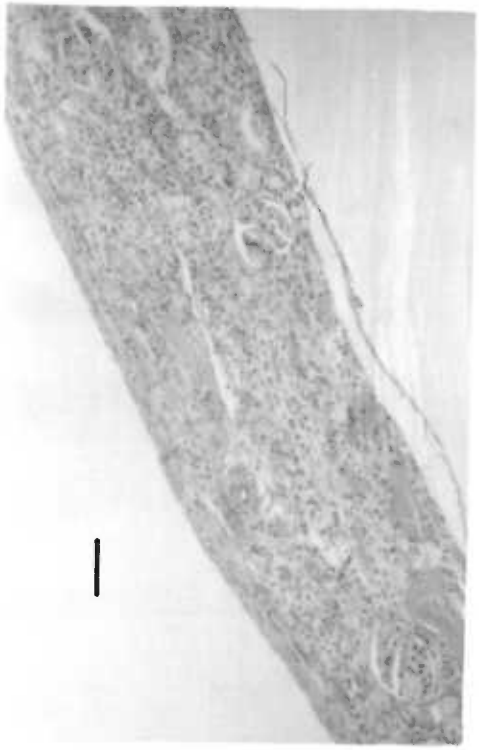
Photograph 18: Sprague-Dawley rat number 77-104 6 days after surgical implantation of catheters using the old technique for catheterization of the bladder. Tubular degeneration.

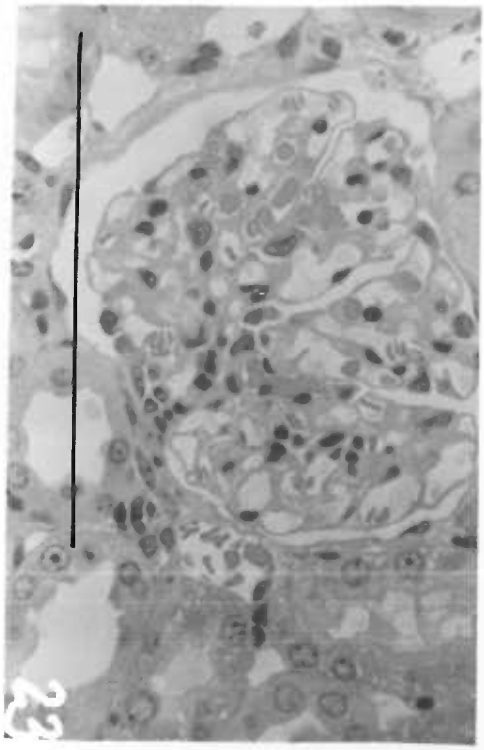
Photograph 22: SHR rat number 77-106 2 days after the surgical implantation of catheters using the old technique for catheterization of the bladder. Mild arteriolar hyalinization.

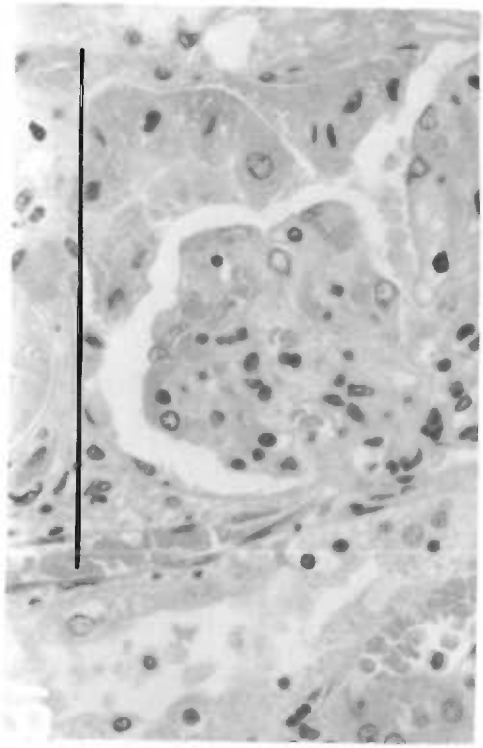
Photograph 23: WKY rat number 78-99 31 days after the surgical implantation of catheters using the new technique for catheterization of the bladder. Mild vacuolization of the renal tubules.

Bars on photographs are 100 microns.









RENAL FUNCTION CURVES

Examples of Pressure Records

Although the arterial pressure record was of acceptable fidelity immediately after the catheter was placed in the aorta, usually I was unable to obtain a reliable record of the magnitude of arterial pulsations several days after surgery. Figure 41 is an illustration of an exceptionally good record obtained just minutes after the catheter was put into the aorta. Even so, the dichrotic notch is not well-defined in this record.

Figure 42 shows two examples of records from experiments in which there was only one urine collection period. For rat number 78-88, the mean arterial pressure during the collection period was 103.5 $\pm$ 5.2 (SD) mmHg. This rat was sleeping during part of the experiment. This was not an unusual finding; most rats remained unexcited during the experimental periods. In a few cases, the rats became disturbed while I was flushing their bladder. Data from these rats were discarded because leaks were found at the junction between the catheter and bladder at the post-mortem examination of these rats.

Figure 43 has two examples of pressure and heart rate records from experiments in which there were two urine collection periods on the same day. In both of these cases I withdrew blood between the first and second periods in order to reduce the blood pressure but there were also many experiments in which I infused blood from a donor rat between the collections periods in order to increase the blood pressure for the second period.

The increase in the heart rate of rat number 78-134 after the infusion of hexamethonium was seen in many other rats and is readily

explained on the basis of vagal blockade.

Both records in Figure 43 also illustrate the trend for blood pressure to decrease during the experimental period. It is not clear whether this is due to impaired myocardial function secondary to prolonged hypotension or to a progressive depletion of adrenergic neurotransmitters in the heart after ganglionic blockade.

Figure 41: An example of a good arterial pressure recording obtained immediately after the implantation of a catheter in the abdominal aorta.

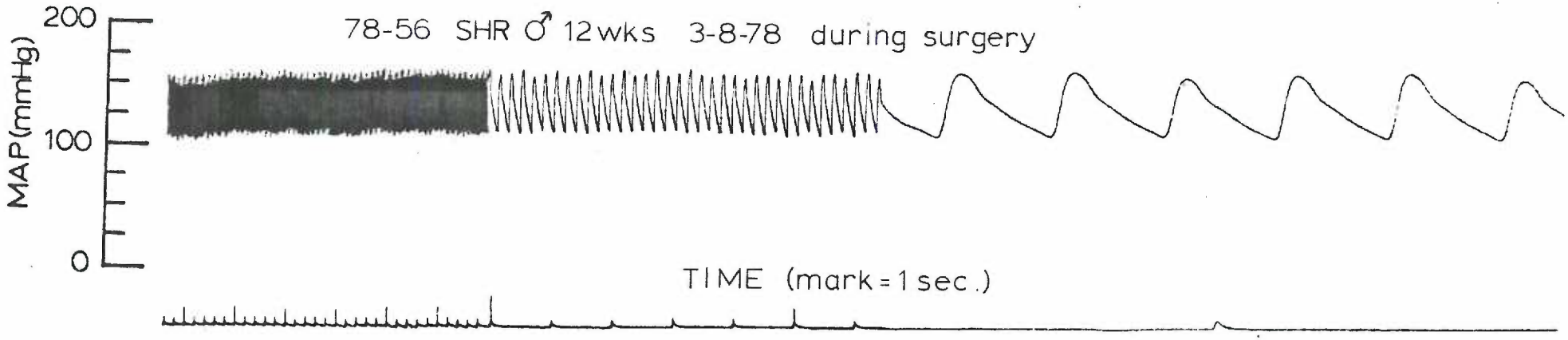

78-56 SHR ♂ 12wks 3-8-78 during surgery
MAP(mmHg)
200
100
0
TIME (mark = 1 sec.)

Figure 42: Recordings of mean arterial pressure (MAP)and heart rate for two rats during acute experimental procedures.

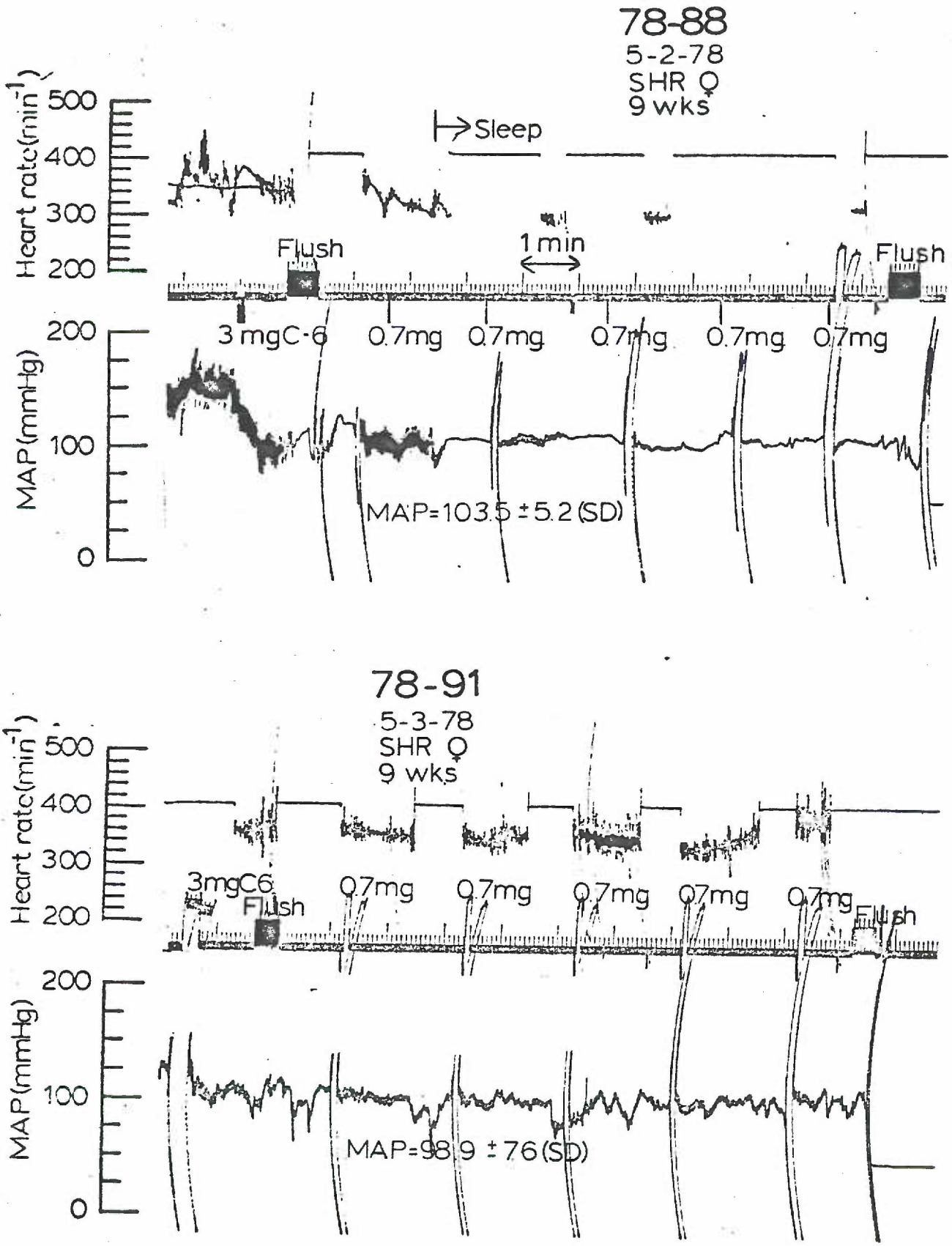
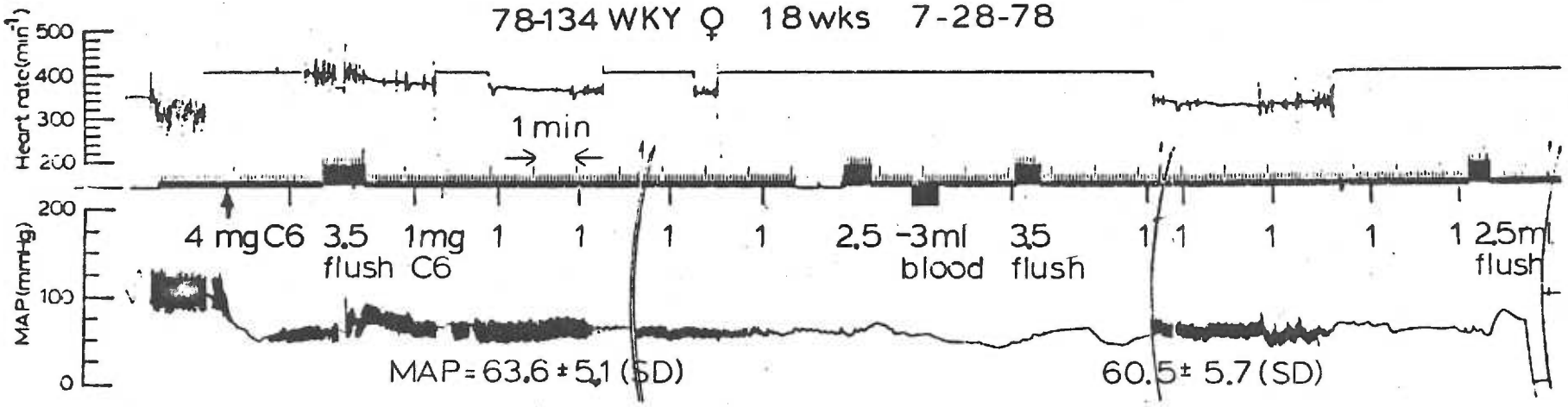
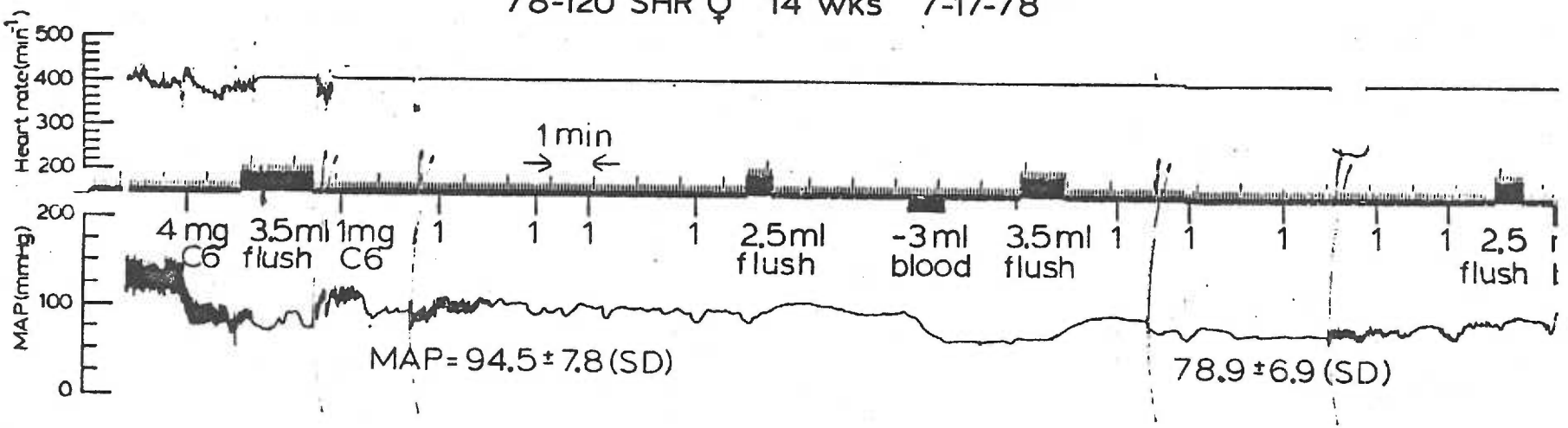

78-88
5-2-78
SHR ♀
9 wks
Heart rate(min⁻¹)
500
400
300
200
Sleep
Flush
1 min
Flush
3 mgC-6
0.7mg
0.7mg
0.7mg
0.7mg
0.7mg
MAP(mmHg)
200
100
0
MAP=103.5 ± 5.2 (SD)
78-91
5-3-78
SHR ♀
9 wks
Heart rate(min⁻¹)
500
400
300
200
3mgC6
Flush
0.7mg
0.7mg
0.7mg
0.7mg
0.7mg
Flush
MAP(mmHg)
200
100
0
MAP=98.9 ± 7.6 (SD)

Figure 43: Recordings of mean arterial pressure (MAP) and heart rate for two rats during acute experimental procedures.


78-134 WKY ♀ 18 wks 7-28-78
Heart rate(min⁻¹)
500
400
300
200
1 min
MAP(mmHg)
200
100
0
4 mg C6
3.5 flush
1mg C6
1
1
1
1
2.5
-3ml blood
3.5 flush
1
1
1
1
1
2.5ml flush
MAP = 63.6 ± 5.1 (SD)
60.5 ± 5.7 (SD)


78-120 SHR ♀ 14 wks 7-17-78
Heart rate(min⁻¹)
500
400
300
200
1 min
MAP(mmHg)
200
100
0
4 mg C6
3.5ml flush
1mg C6
1
1
1
1
2.5ml flush
-3ml blood
3.5ml flush
1
1
1
1
1
2.5 flush
MAP = 94.5 ± 7.8 (SD)
78.9 ± 6.9 (SD)

The Renal Function Curves

Figure 44 depicts renal function curve data from one representative SHR rat and one WKY rat of comparable age. The results of renal function curves will be easier to interpret, however, if data from all rats are included in the analysis. I have analyzed the data relating mean arterial pressure and sodium excretion rate after ganglionic blockade in two ways.

First, I have treated each measurement as an independent observation and plotted the points representing mean arterial pressure and sodium excretion rate to obtain a renal function curve after ganglionic blockade. Figures 45, 46, 47 are examples of this type of analysis.

In the second method of analysis, I treated each rat as an independent observation. Some rats survived with fully functional catheters and normal indices of kidney function for several weeks after surgery and I was able to get up to 15 measurements of arterial pressure and sodium excretion rate from one rat. In other rats, I was able to measure these parameters only once. By taking the mean of all arterial pressures and the mean of all sodium excretion rates measured in a given rat, I obtained one point on the renal function curve from each rat. Figures 48, 49 and 50 were developed from these data.

It is not entirely appropriate to treat each observation as independent as I have done in the first analysis since the several observations from a single rat were not independent. The second type of analysis is more defensible statistically but much of the information on the nature of the renal function curve is lost by this analysis.

Therefore, I will use the first analysis to gain a general understanding of the renal function curve but I will use the second analysis as the basis for statements based on statistical analysis.

Note that, at all ages and by both types of analysis, the renal function curves for SHR rats are clearly to the right of the curves for WKY rats. In each case, I calculated the linear regression line which minimized the square of the horizontal or the pressure deviation from the regression line.

The Appendix lists all the observations of paired values of arterial pressure and sodium excretion rate.

Figure 44: Renal function curves after ganglionic blockade from a single SHR rat and a single WKY rat. Note that the renal function curves from these rats are similar to the curves which were obtained from the same strain when all the individual observations were used to define the renal function curve (Figure 46) or when each rat was represented by a single point (Figure 49).

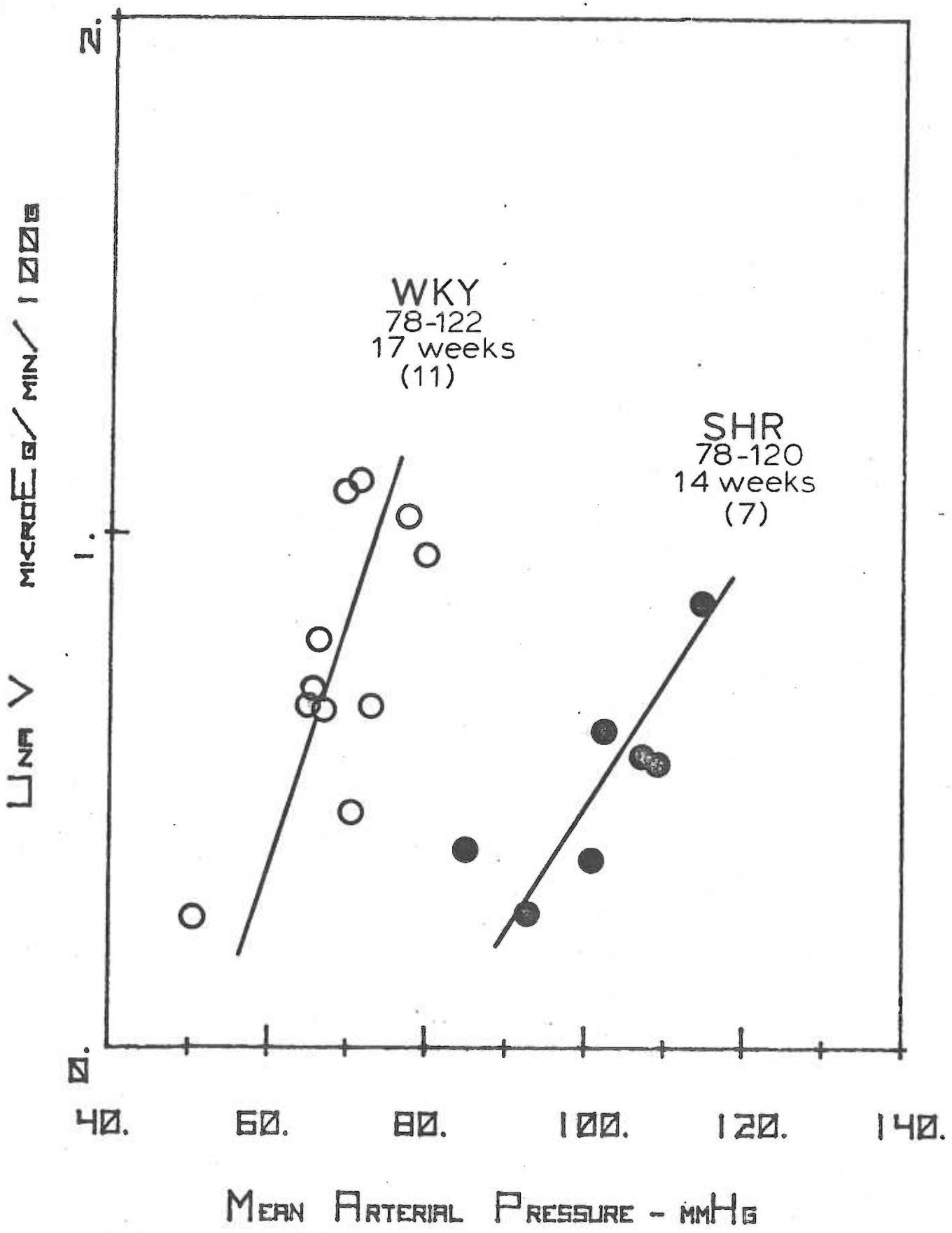

2.
1.
0.
WKY
78-122
17 weeks
(11)
SHR
78-120
14 weeks
(7)
40.
60.
80.
100.
120.
140.
MEAN ARTERIAL PRESSURE - MMHG
UNA V MICROEQ/MIN/100G

Figure 45: Renal function curves after ganglionic blockade which were obtained by using all the individual observations from 8 to 12 week old SHR and WKY rats. (n) = number of rats.

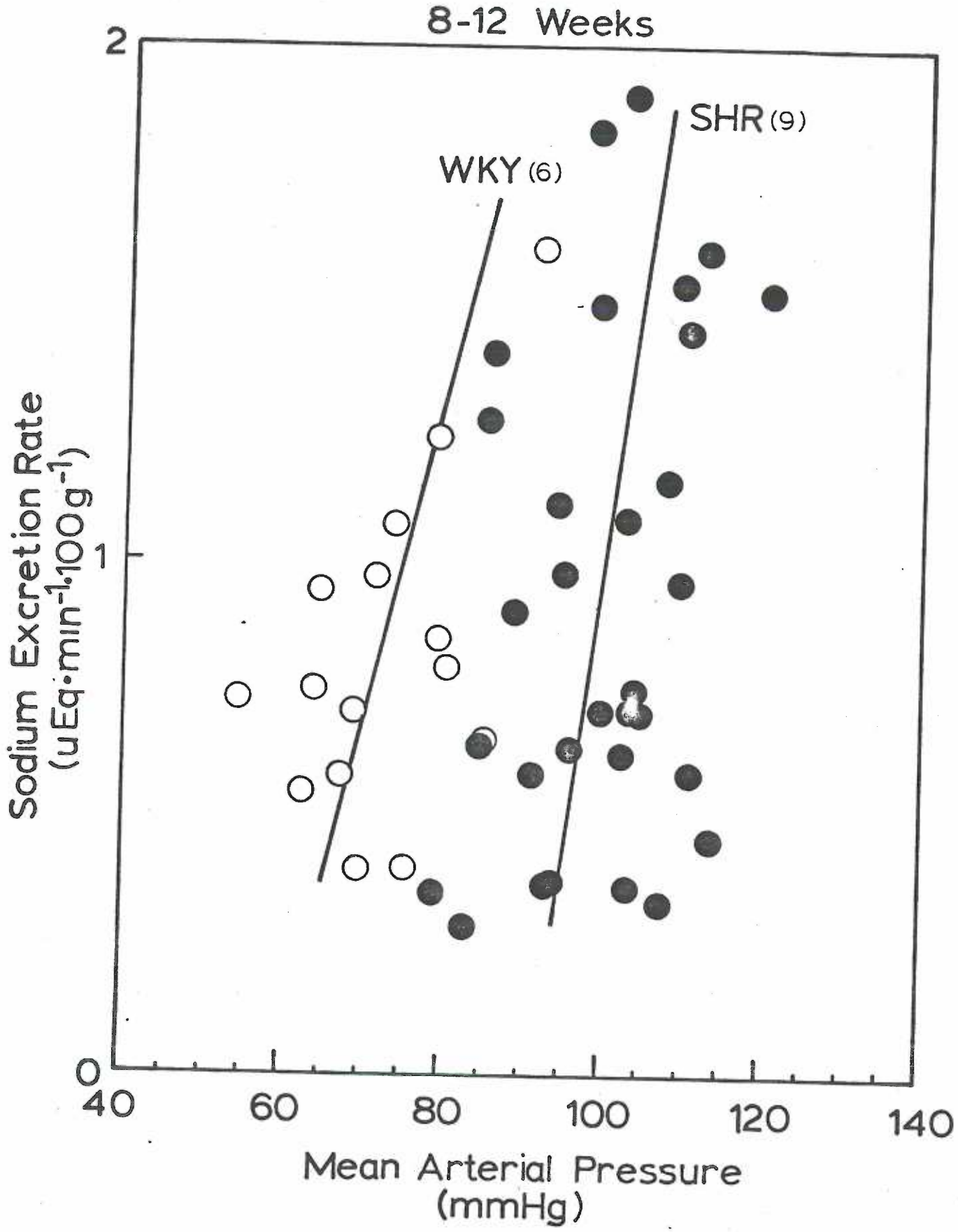

8-12 Weeks
SHR (9)
WKY (6)
Sodium Excretion Rate
(uEq·min⁻¹·100g⁻¹)
2
1
0
40
60
80
100
120
140
Mean Arterial Pressure
(mmHg)

Figure 46: Renal function curves after ganglionic blockade which were obtained by using all the individual observations from 14 to 19 week old SHR and WKY rats. (n) = number of rats.

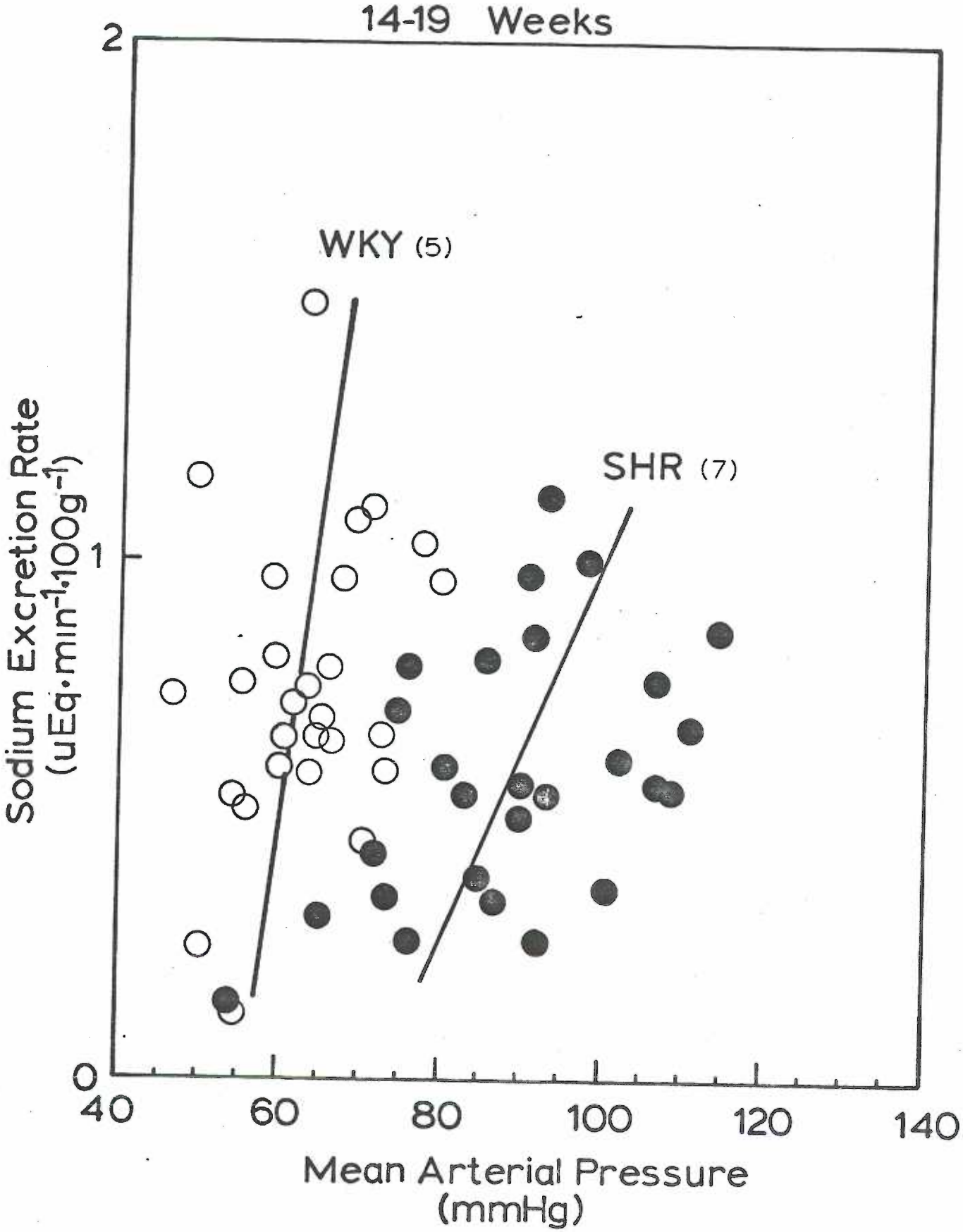

14-19 Weeks
WKY (5)
SHR (7)
Sodium Excretion Rate
(uEq·min⁻¹·100g⁻¹)
2
1
0
40
60
80
100
120
140
Mean Arterial Pressure
(mmHg)

Figure 47: Renal function curves after ganglionic blockade which were obtained by using all the individual observations from 20 to 32 week old SHR and WKY rats. (n) = number of rats.

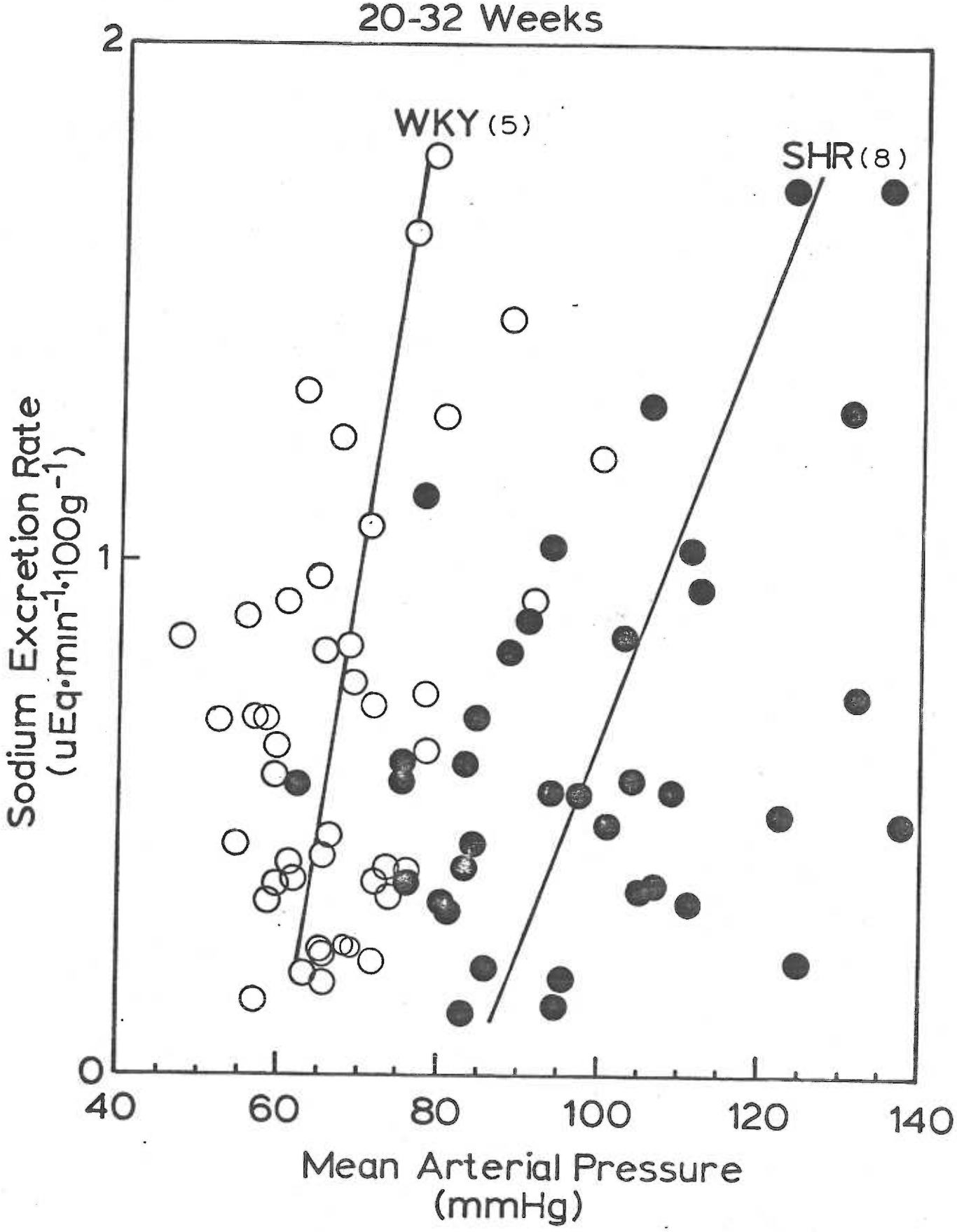

20-32 Weeks
WKY (5)
SHR (8)
Sodium Excretion Rate
(uEq·min⁻¹·100g⁻¹)
2
1
0
40
60
80
100
120
140
Mean Arterial Pressure
(mmHg)

Figure 48: Renal function curves after ganglionic blockade which were obtained by using one point to represent the mean of the observations from a given rat. Data are for SHR and WKY rats from 8 to 12 weeks old.

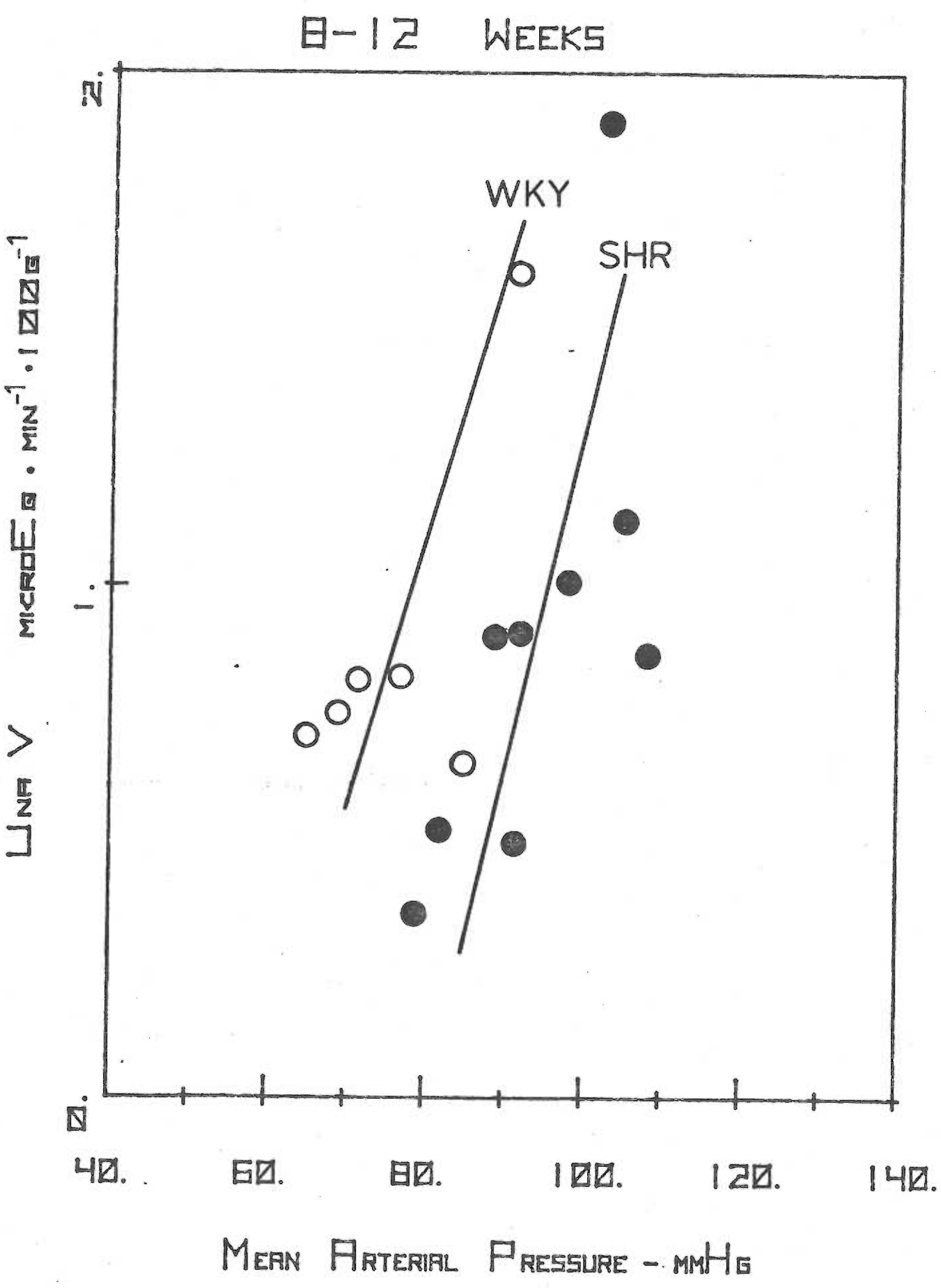

8-12 WEEKS
2.
1.
0.
WKY
SHR
$U_{NA}V$ MICROEQ · MIN^{-1} · 100G^{-1}
40.
60.
80.
100.
120.
140.
MEAN ARTERIAL PRESSURE - MMHG

Figure 49: Renal function curves after ganglionic blockade which were obtained by using one point to represent the mean of the observations from a given rat. Data are for SHR and WKY rats from 14 to 19 weeks old.

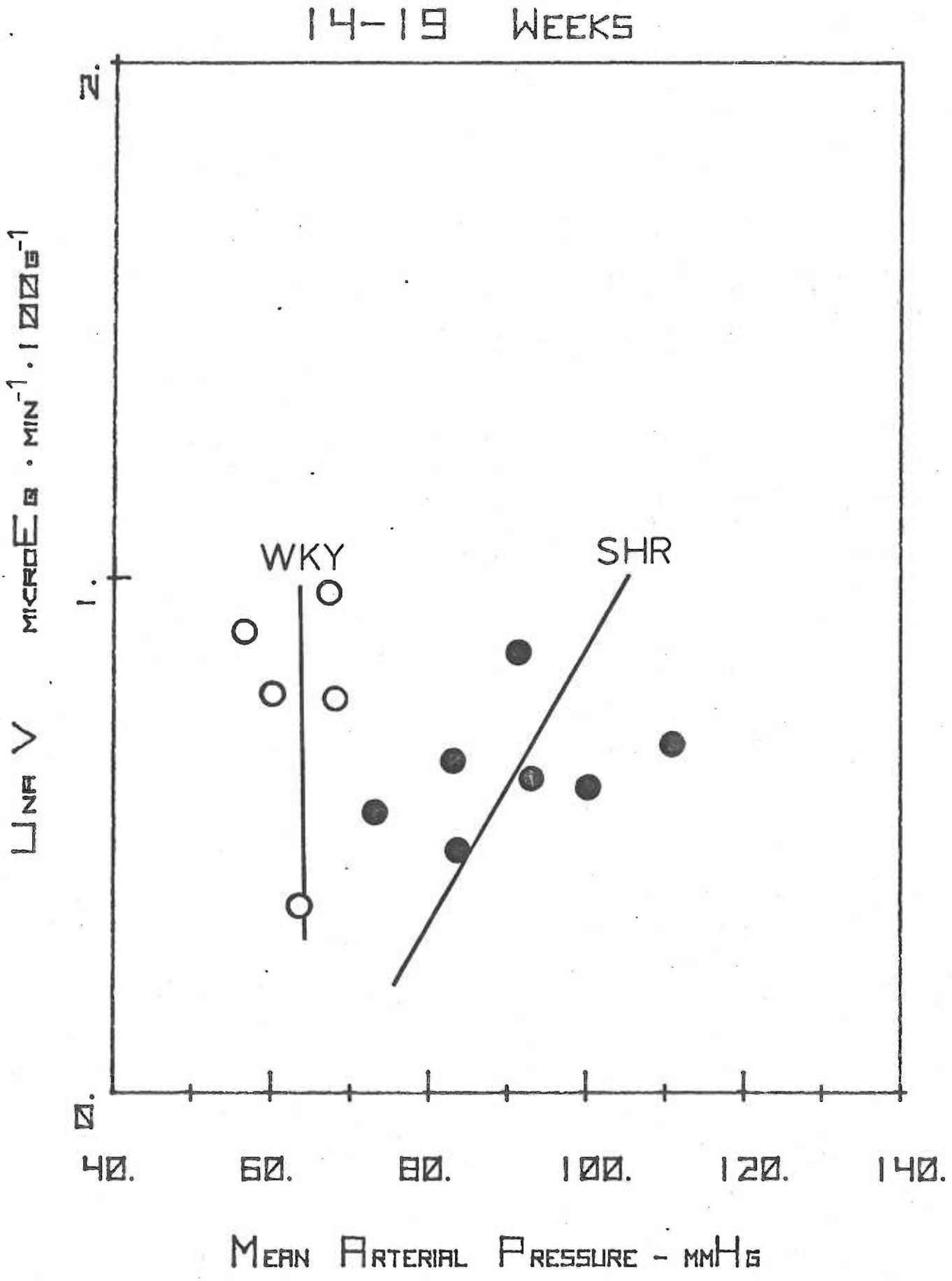
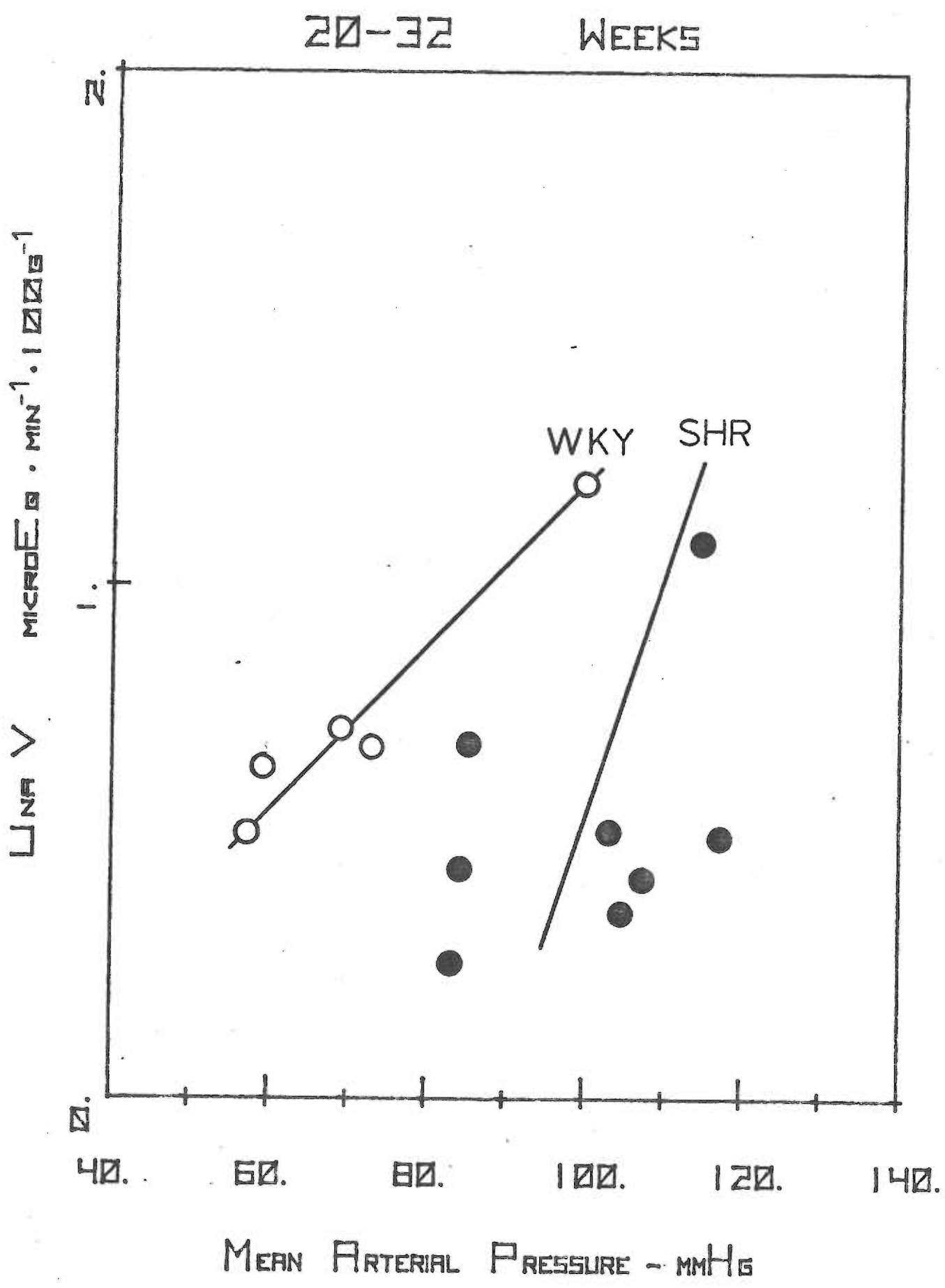

14-19 WEEKS
2.
1.
0.
WKY
SHR
$U_{NA}V$ MICROEQ · MIN^{-1} · $100G^{-1}$
40.
60.
80.
100.
120.
140.
MEAN ARTERIAL PRESSURE - MMHG

Figure 50: Renal function curves after ganglionic blockade which were obtained by using one point to represent the mean of the observations from a given rat. Data are for SHR and WKY rats from 20 to 32 weeks old.


20-32 WEEKS
2.
1.
0.
WKY
SHR
40.
60.
80.
100.
120.
140.
MEAN ARTERIAL PRESSURE - MMHG
U NA V MICROEQ · MIN-1 · 100G-1

Comparison between Groups

Table 9 presents a summary of the linear regression lines which represent the renal function curves for the cases in which each observation was treated independently and in which the observations from the same rat were combined to give a mean value for arterial pressure and sodium excretion rate for that rat.

Let us first examine the renal function curves that were determined with each observation treated independently. The sample correlation coefficient (r) was not significant for some groups. Figure 51 shows, however, that the SHR renal function curve was to the right of the age-matched WKY curve for every age group. Furthermore, there was no age-related difference in the curves for either SHR or WKY rats. This feature prompted me to combine all age groups and to analyze the resultant renal function curves for SHR and WKY rats. The linear regression lines representing the renal function curves for all ages combined had significant correlation coefficients and they were subjected to an analysis of variance which tested for a difference between the SHR and WKY curves (Neter and Wasserman, 1974). The lines for the combined SHR and WKY data were significantly different (P 0.005). A difference between two curves which is detected by this test could be due to a difference in the slopes, a difference in the positions of the curves or both. Consequently, I tested for a difference between the slopes of the renal function curves of SHR and of WKY rats. The slopes were not statistically different. From these observations, it appears that the renal function curve after ganglionic blockade for SHR rats is to the right of the

Table 9: Linear regression lines for renal function curves. The linear regression equations are computed so that the sum of the squares of the error in mean arterial pressure (MAP) is minimized. Equations are of the form:

$$U_{Na}V \text{ (microEq·min}^{-1}\text{·100g}^{-1}\text{)} = A + B \cdot \text{MAP (mmHg)}$$

Rat type	SHR				WKY			
Age group (weeks)	8-12	14-19	20-32	All*	8-12	14-19	20-32	All
	Each observation treated independently							
n	31	27	38	96	15	28	43	86
A	-15.89	-2.68	-3.62	-5.62	-4.01	-6.92	-6.21	-6.05
B	0.1686	0.0368	0.0424	0.0653	0.0668	0.1223	0.1018	0.1014
r	0.279	0.461	0.414	0.365	0.494	0.302	0.390	0.359
p (for r=0)	> 0.05	< 0.025	< 0.01	< 0.001	> 0.05	> 0.05	< 0.01	< 0.001
	Observations combined for each rat							
n	9	7	8		6	5	5	
A	-5.31	-1.80	-4.15		-3.05	62.65	-0.37	
B	0.0658	0.0267	0.0468		0.0516	-0.977	0.0157	
r	0.456	0.133	0.152		0.465	0.002	0.945	
p (for r=0)	> 0.05	> 0.05	> 0.05		> 0.05	> 0.05	< 0.005	

* SHR curve is different from the WKY curve ($P < 0.005$).

curve for age-matched WKY rats at all ages tested.

While there was no statistical difference in the positions of the renal function curves for different age groups of the same strain, there was a tendency for the horizontal separation of SHR and WKY curves to increase with age. Table 10 summarizes these data. The horizontal separation of the renal function curves at the mean chronic sodium excretion rate was computed from the regression lines obtained when each observation was treated independently. I chose to compare the curves at the chronic sodium excretion rate since this was the sodium excretion rate at which I was able to compare the chronic renal function curves. Further, for a curve defined by the linear regression technique which I used, the variance of the position of the curve is least at the mean value of the parameters. The mean value of acute sodium excretion rate is similar to the value of the chronic sodium excretion rate in my experiments.

Note that the separation of the renal function curves after ganglionic blockade was essentially the same as the difference in the resting mean arterial pressure between SHR and WKY rats at the same age. One anomalous feature of Figure 51 is that the renal function curve for the 14 to 19 week old rats is to the left of the curve for younger or older age groups. This difference in the position of the curves is not statistically significant but the fact that the 14 to 19 week renal function curve for both SHR and WKY rats was to the left of the curve for other age groups aroused my curiosity.

Since most of the data for 14 to 19 week old rats were collected during the summer months while most of the data for other age groups were collected in the winter and spring, it is possible that a change

Figure 51: Lines representing the renal function curves after ganglionic blockade which were obtained by using all the individual observations from SHR and WKY rats. See Figures 46, 47 and 48 for the data points.

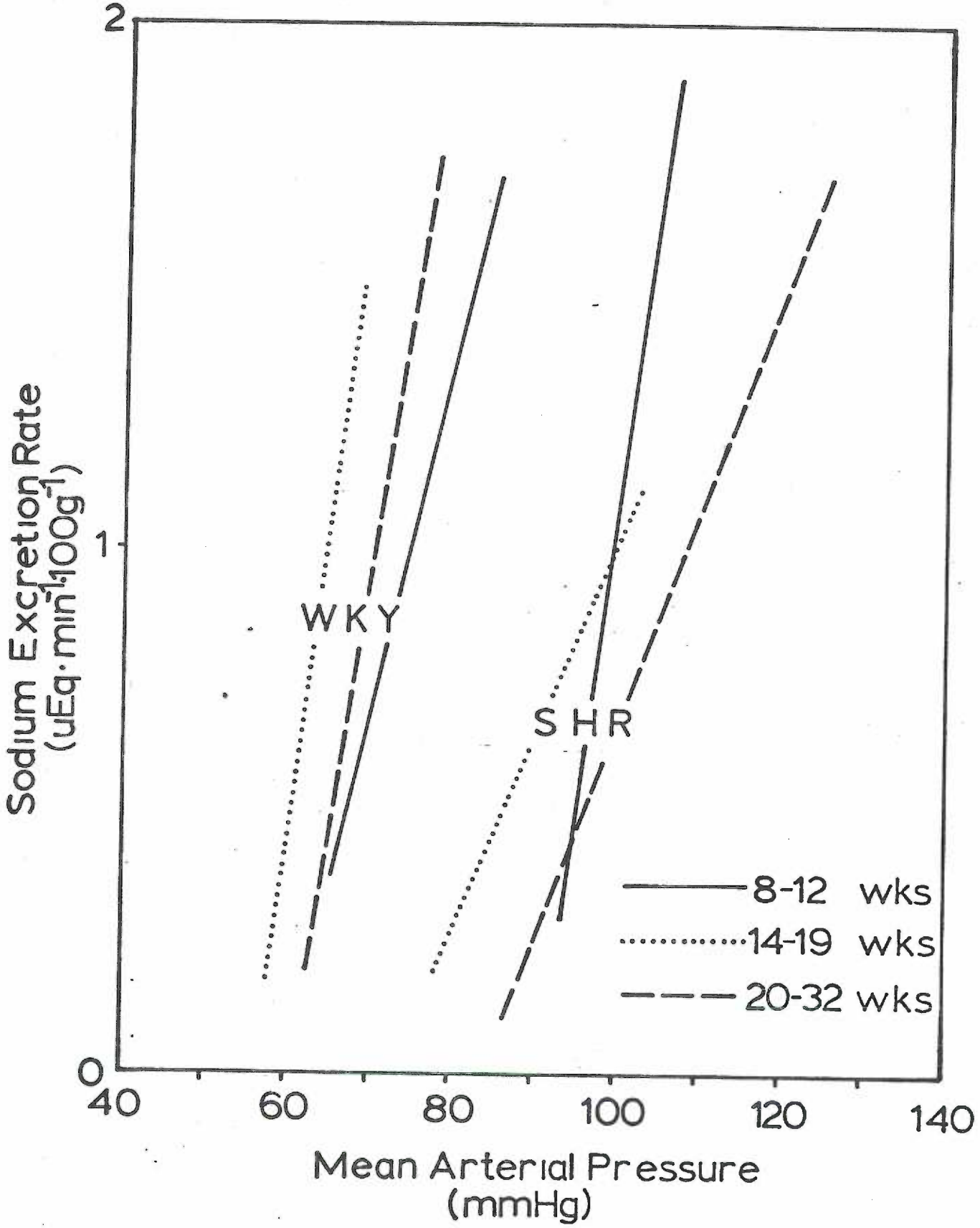

2
1
0
Sodium Excretion Rate
(uEq·min⁻¹·100g⁻¹)
WKY
SHR
8-12 wks
14-19 wks
20-32 wks
40
60
80
100
120
140
Mean Arterial Pressure
(mmHg)

Table 10: The differences in the chronic mean arterial pressures (MAP) between SHR and WKY rats and the differences in the horizontal separation (pressure difference) between the renal function curves after ganglionic blockade for SHR and WKY rats. The horizontal separation was computed at the chronic sodium excretion rate ($U_{Na}V$). Data are presented as the mean ± SEM. (n) = number of observations.

8 to 12 weeks	SHR	WKY	Difference (SHR-WKY)
Resting MAP (mmHg)	122.6±3.2 (13)	99.8±2.6 (10)	22.8±4.1
Pressure (mmHg) computed from experimental renal function curves at the chronic $U_{Na}V$	99.6±1.8 (31)	73.5±2.4 (15)	26.1±3.0
Chronic $U_{Na}V$ ($microEq \cdot min^{-1} \cdot 100g\ BW^{-1}$)	0.96	0.84	
14 to 19 weeks			
Resting MAP (mmHg)	136.2±3.5 (12)	102.7±2.4 (12)	33.5±4.2 **
Computed pressure (mmHg)	95.5±3.6 (27)	63.4±1.5 (28)	32.1±3.9 *
Chronic $U_{Na}V$	0.80	0.86	
20 to 32 weeks			
Resting MAP (mmHg)	146.2±3.2 (11)	111.3±1.3 (6)	34.9±3.5 **
Computed pressure (mmHg)	104.7±3.5 (38)	69.1±1.6 (43)	35.6±3.8 **
Chronic $U_{Na}V$	0.89	0.75	

* Different from the difference at 8 to 12 weeks ($P < 0.05$).
** Different from the difference at 8 to 12 weeks ($P < 0.01$).

in body fluid volume related to the ambient temperature was responsible for the relative leftward position of the curves for 14 to 19 week old rats. When I moved the water columns which I used to calibrate arterial pressure readings on June 1, 1978 I may have introduced a systematic error to all subsequent readings. Figure 52 is a plot of the arterial pressures recorded during the summer months and during the winter months in relation to the age of the rats. No seasonally-related difference is apparent. The interpretation of the leftward position of the renal function curves at 14 to 19 weeks for both SHR and WKY rats remains in doubt.

Let us now examine the renal function curves after ganglionic blockade as they were determined when the observations from any one rat were combined to give a mean sodium excretion rate and a mean arterial pressure after ganglionic blockade for that rat. With one exception, these renal function curves (Table 9 and Figure 53) do not have sample correlation coefficients that differ significantly from zero. Thus, I have not applied an analysis to the linear regression lines. Instead, I have used a simpler method to analyze the difference between the groups.

By an analysis of variance of the data from all rats within a given group, I found that there was not a significant difference in the mean sodium excretion rate after ganglionic blockade among the groups (Figure 54). There was, however, a significant difference between the mean arterial pressure after ganglionic blockade of SHR and age-matched WKY rats (Figure 55). There was no difference in the post-blockade mean arterial pressure between the various age groups of the same strain. Since the arterial pressures of SHR rats were greater than those for WKY

Figure 52: Mean arterial pressures recorded from conscious unrestrained rats of various ages. The data include pressures recorded before and after June 1, 1978.

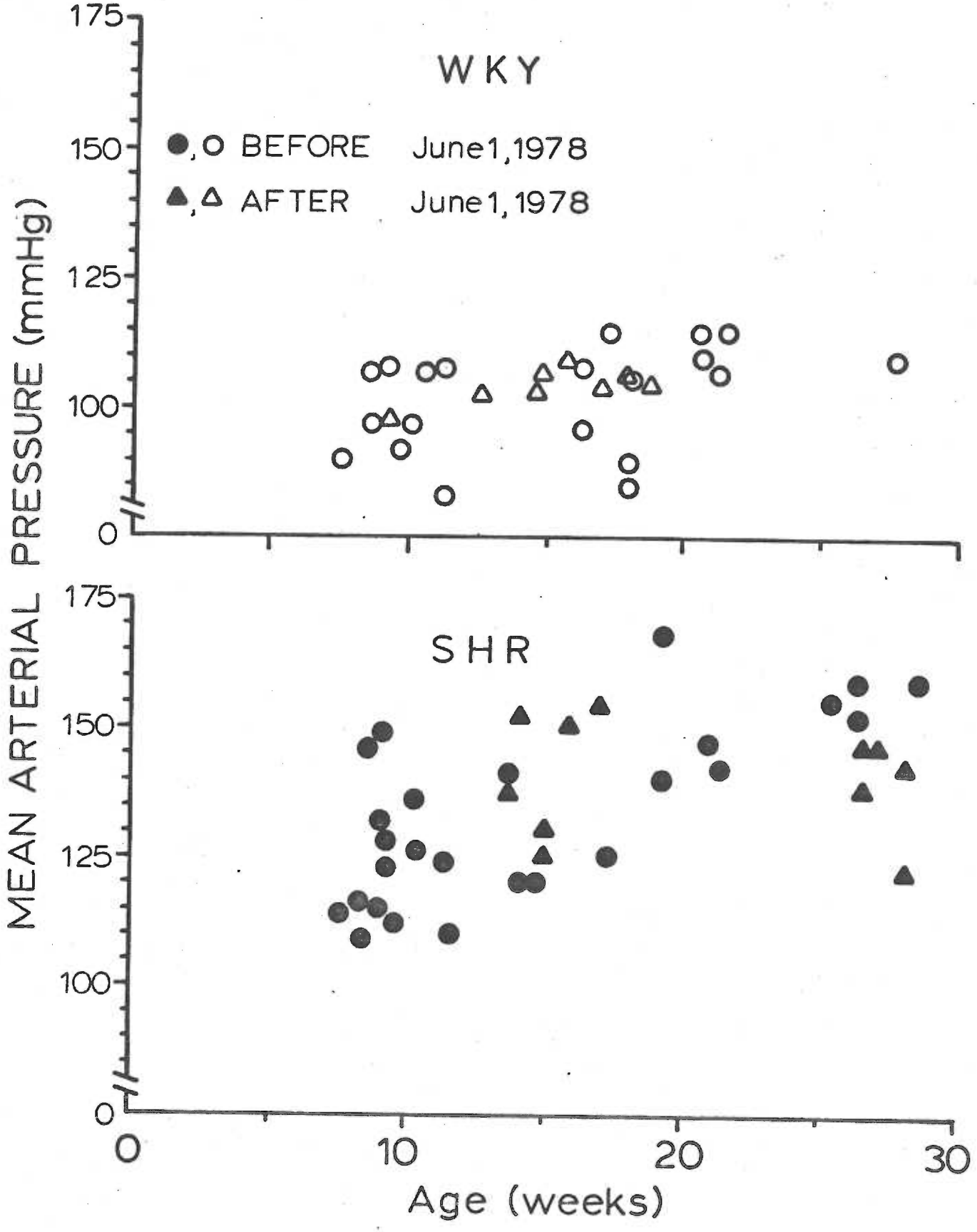

WKY
●,○ BEFORE June1,1978
▲,△ AFTER June1,1978
SHR
MEAN ARTERIAL PRESSURE (mmHg)
175
150
125
100
0
0
10
20
30
Age (weeks)

Figure 53: Lines representing the renal function curves after ganglionic blockade which were obtained by using one point to represent the mean of the observations from a given rat. See Figures 49, 50 and 51 for the data points.

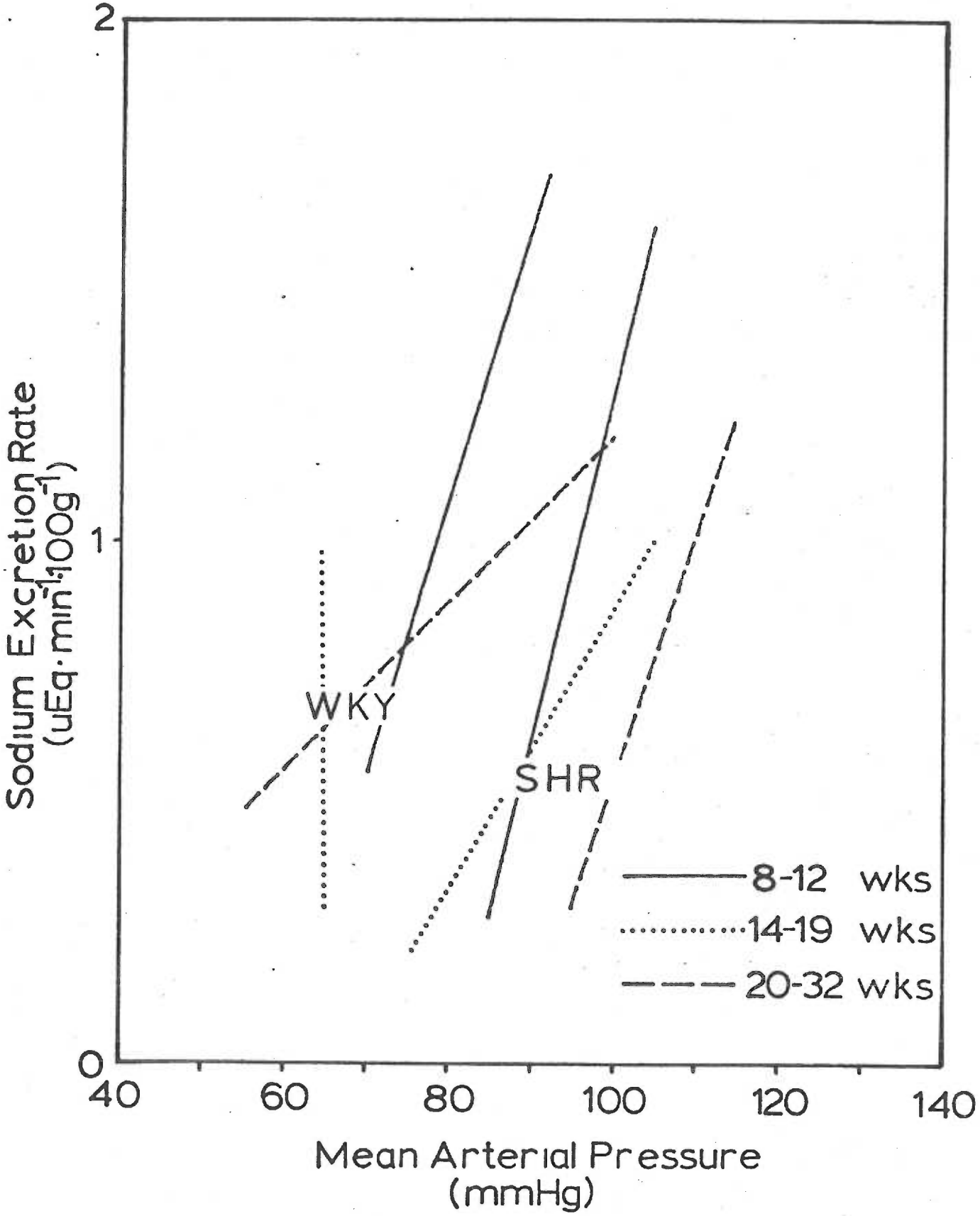
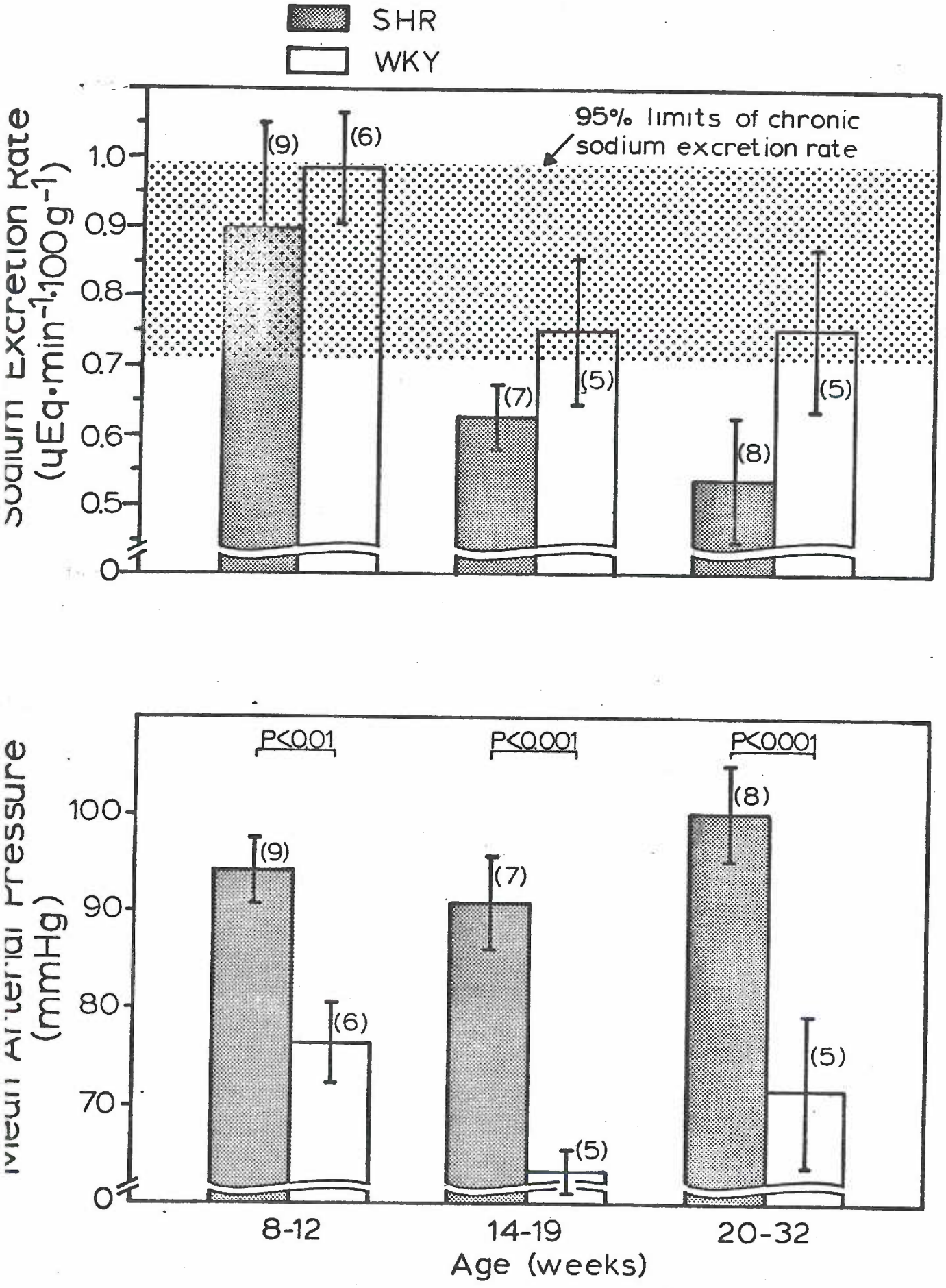

2
1
0
40
60
80
100
120
140
Sodium Excretion Rate
(uEq·min⁻¹·100g⁻¹)
Mean Arterial Pressure
(mmHg)
WKY
SHR
8-12 wks
14-19 wks
20-32 wks

Figure 54: Sodium excretion rate after ganglionic blockade for SHR and WKY rats. Values are given as the mean ± SEM. The stippled band gives the 95% limits for sodium excretion rate measured with 24-hour urine collections from SHR and WKY rats without hexamethonium.

Figure 55: Mean arterial pressure after ganglionic blockade for SHR and WKY rats. Values are given as the mean ± SEM.


SHR
WKY
95% limits of chronic sodium excretion rate
Sodium Excretion Rate (μEq·min⁻¹·100g⁻¹)
1.0
0.9
0.8
0.7
0.6
0.5
0
(9)
(6)
(7)
(5)
(8)
(5)
P<0.01
P<0.001
P<0.001
Mean Arterial Pressure (mmHg)
100
90
80
70
0
(9)
(6)
(7)
(5)
(8)
(5)
8-12
14-19
20-32
Age (weeks)

rats at the same sodium excretion rats, these data provide evidence that, during the period of developing hypertension in the SHR rat, the renal function curve after ganglionic blockade is to the right of the curve for age-matched WKY rats.

Many reports use kidney weight rather than body weight to normalize sodium excretion rate. Am I justified in using body weight to normalize sodium excretion rate in this work and is it valid to compare my results with those studies which used kidney weight for normalization?

Figure 56 summarizes the ratio of kidney weight to body weight for SHR and WKY female rats in relation to body weight. Two features are important. First, SHR and WKY rats have identical ratios of kidney weight to body weight. Second, there is a significant inverse relation between this ratio and the body weight. When I used the regression line in Figure 57 to normalize the sodium excretion rates which are presented in Figure 55, I again found that there was no statistical difference between the sodium excretion rates for the groups. This conclusion is summarized in Table 11.

Figure 56: The ratio of the weight of both kidneys to body weight (g/100g) for male and female SHR and WKY rats weighing from 80 g to 380 g.

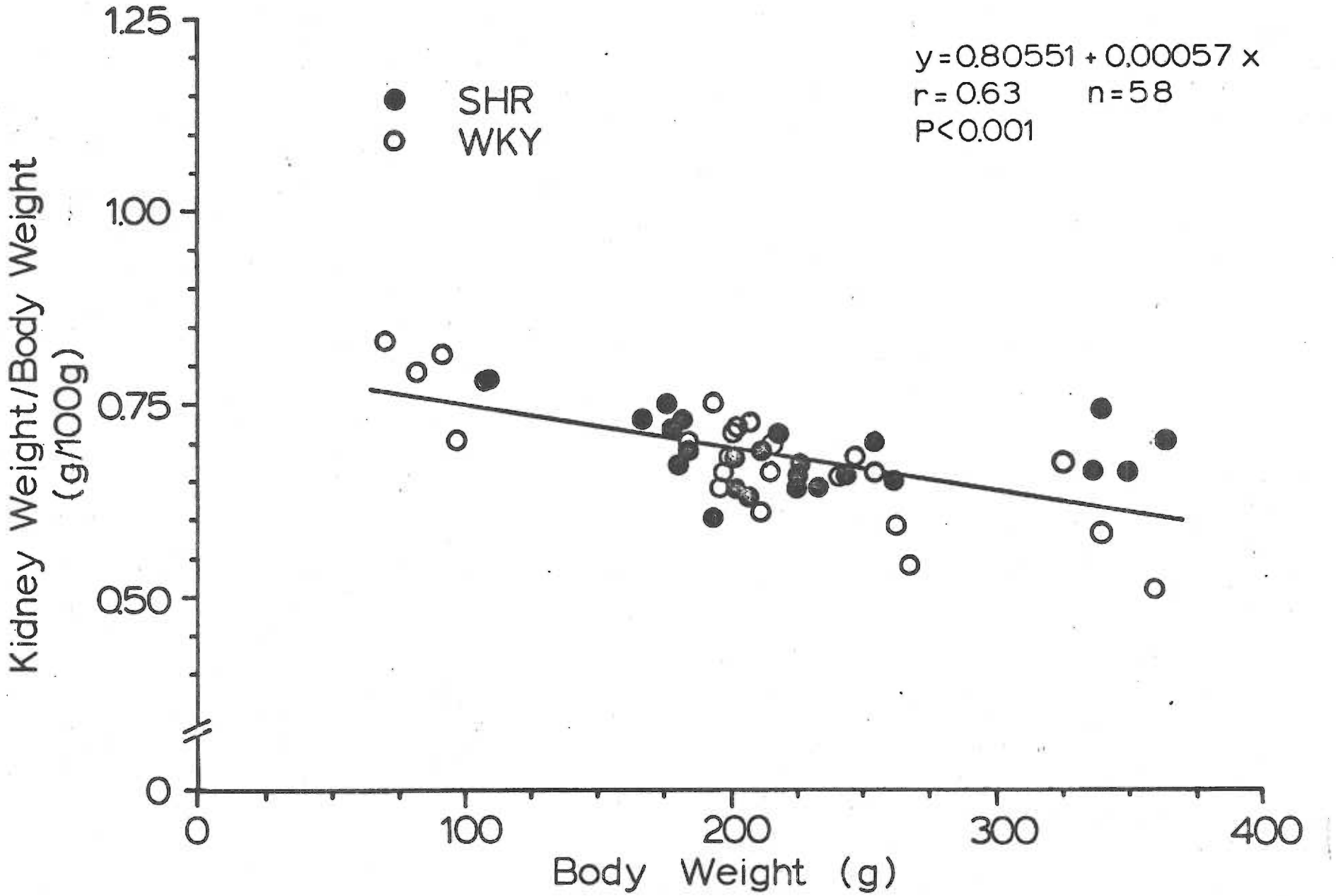

y = 0.80551 + 0.00057 x
r = 0.63 n = 58
P < 0.001
SHR
WKY
Kidney Weight/Body Weight (g/100g)
1.25
1.00
0.75
0.50
0
0
100
200
300
400
Body Weight (g)

Table 11: Comparison of sodium excretion rate ($U_{Na}V$ microEq•min^{-1}) normalized to body weight (100g BW^{-1}) or to kidney weight (g KW^{-1}). Conversion from body weight to kidney weight normalization was made with the regression line in Figure 56. There is no significant difference in the sodium excretion rates between any of the groups for either of the normalization methods.

	SHR			WKY		
Age (weeks)	8-12	14-19	20-32	8-12	14-19	20-32
Body weight (g)	143.9	177.2	210.7	139.1	200.5	228.9
KW/BW (g/100g)	0.723	0.705	0.685	0.726	0.691	0.675
$U_{Na}V$/100g BW	0.897	0.627	0.537	0.984	0.750	0.754
SEM	± 0.152	± 0.046	± 0.089	± 0.080	± 0.105	± 0.117
$U_{Na}V$/g KW	1.24	0.889	0.784	1.355	1.086	1.117
SEM	± 0.210	± 0.065	± 0.130	± 0.110	± 0.152	± 0.173

COMPARISON OF ACUTE AND CHRONIC RENAL EXCRETION

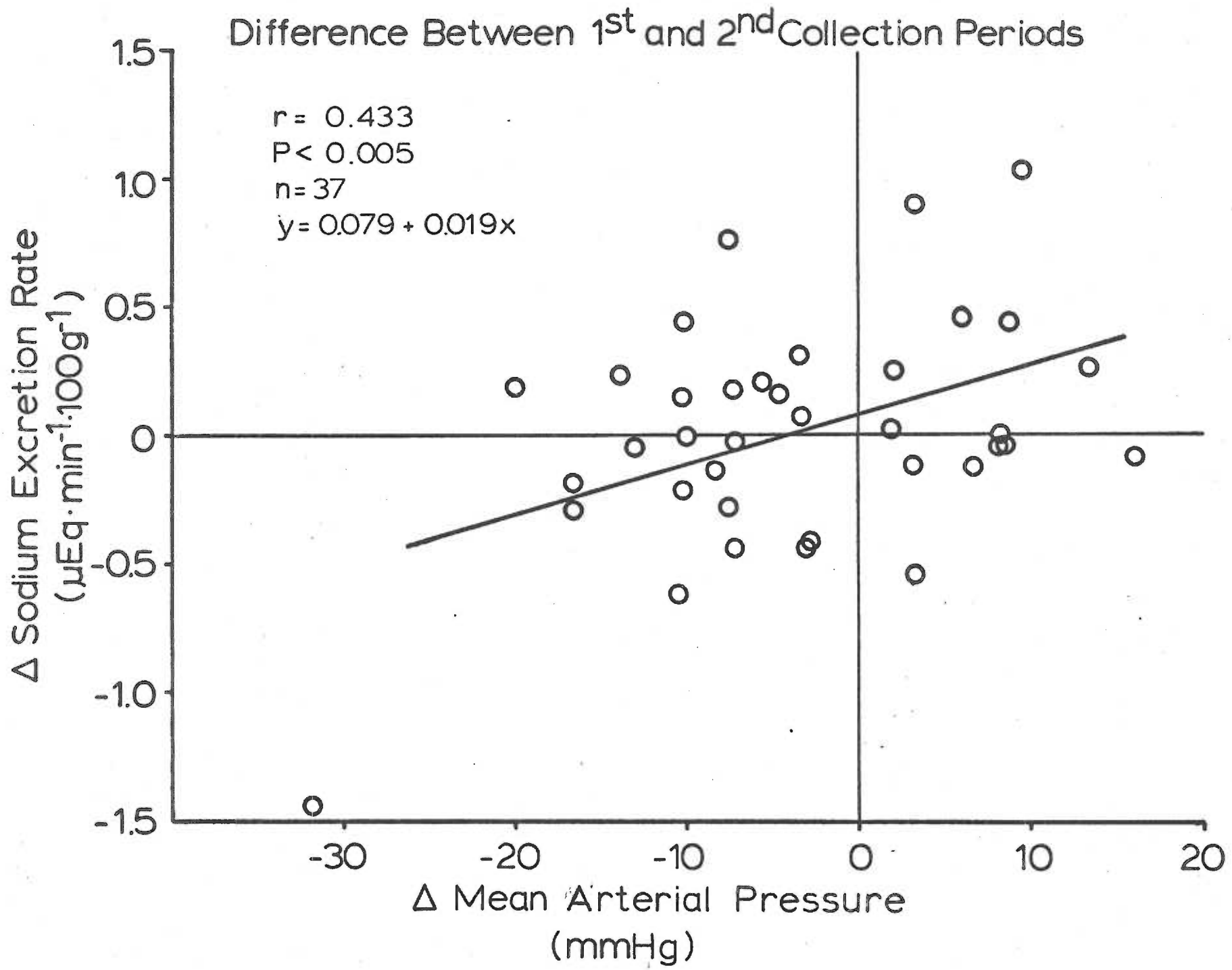
Sodium Excretion Rate

Figure 54 (p 174) reveals not only that there was no difference between groups in sodium excretion rate measured during ganglionic blockade, but also that there was no difference between those sodium excretion rates measured during acute experiments and the rates measured from 24-hour urine collections. This raises the possibility that, because of the time involved in the passage of fluid from the glomerulus to the bladder, the sodium excretion rates which I measured during the acute experimental periods were not representative of the steady state.

While it was clear that there was a positive correlation between sodium excretion rate and mean arterial pressure when all the data are combined, it is important to verify that, with an increase or decrease in blood pressure in an individual rat, there was an appropriate increase or decrease in sodium excretion rate. Figure 57 was derived from data of those rats from which a second urine collection period after hexamethonium blockade was obtained. The change in the mean arterial pressure from the first to the second collection period is plotted against the corresponding change in the sodium excretion rate between the first and the second periods. For instance, if both the arterial pressure and the sodium excretion rate increased from the first to the second collection period, the point would appear in the first quadrant. While there was a great deal of variability, the correlation coefficient was significant ($P < 0.005$). The average change in arterial pressure was significantly less than zero (-3.6 ± 1.7 SEM mmHg) but the change in sodium

excretion rate (0.01 ± 0.07 SEM microEq$\cdot$min$^{-1}\cdot$100g^{-1}) was not different from zero. This indicates that, in the absence of a change in arterial pressure, there would be a tendency for sodium excretion rate to increase from the first to the second collection periods.

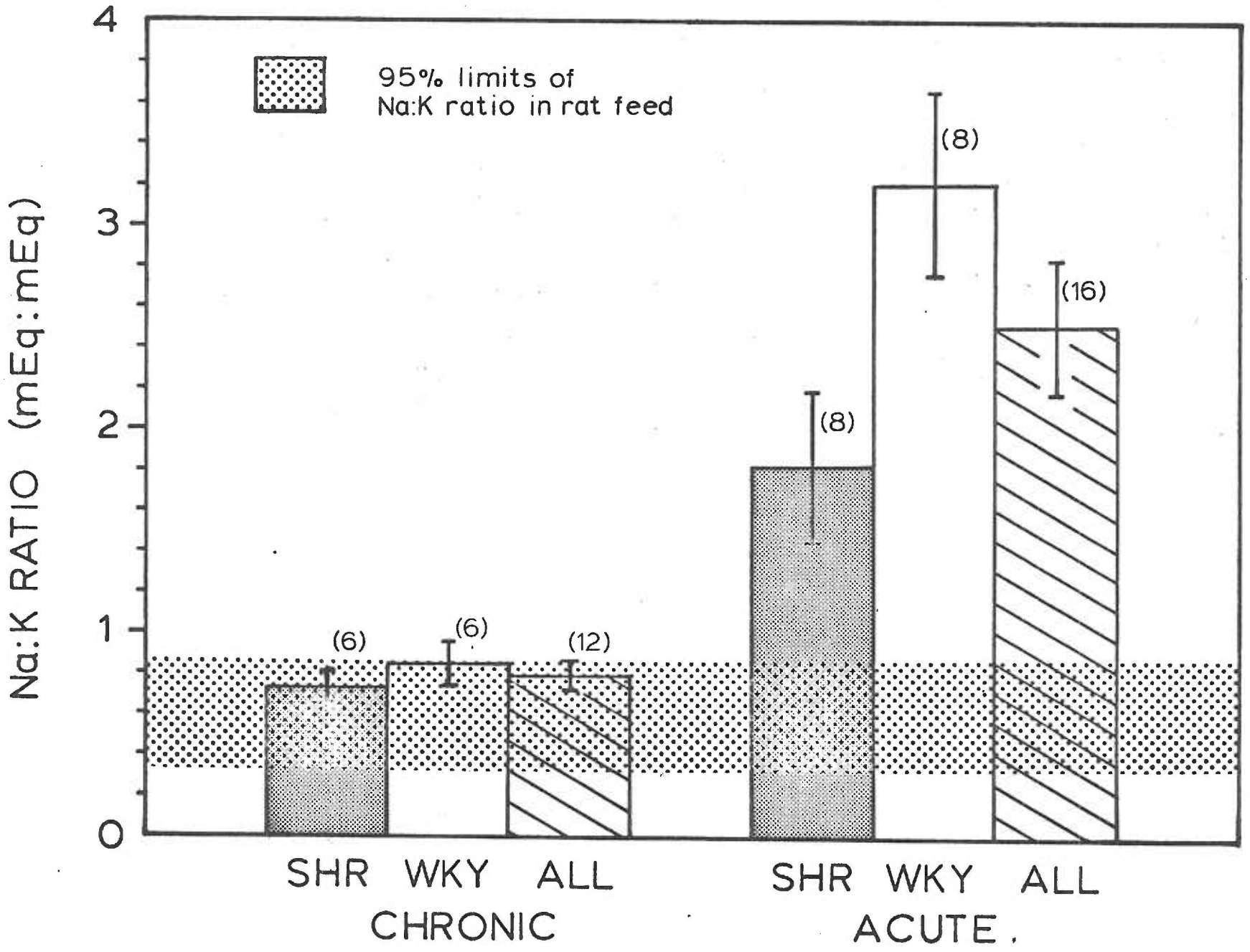
Figure 57: Correlation between the change in mean arterial pressure from the first to the second urine collection periods and the change in sodium excretion rate from the first to the second collection period. Data are from female SHR and WKY rats.


Difference Between 1st and 2nd Collection Periods
r = 0.433
P < 0.005
n = 37
y = 0.079 + 0.019x
Δ Sodium Excretion Rate
(μEq·min-1·100g-1)
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
-30
-20
-10
0
10
20
Δ Mean Arterial Pressure
(mmHg)

Na:K Ratio

I examined the Na:K ratio (mEq:mEq) in urine to gain a better understanding of the differences or similarities between acute and chronic sodium excretion rates. Figure 58 reveals a highly significant difference ($P < 0.001$) between the Na:K ratios measured during acute experiments and the ratios obtained from chronic 24-hour urine collections. There was no difference between Na:K ratios of SHR and of WKY rats for either acute or chronic urine collections. Note also that the Na:K ratios measured with 24-hour urine collections are the same as the Na:K ratio in the rat chow.

Figure 58: Ratio of sodium concentration to potassium concentration in urine from SHR and WKY rats. Chronic urine collections were made for 24-hours with conscious unrestrained rats. Acute urine collections were made after the infusion of hexamethonium. Values for the Na:K ratio are given as the mean ± SEM. (n) = number of rats. The stippled bar indicates the Na:K ratio in the rat feed.


95% limits of
Na:K ratio in rat feed
Na:K RATIO (mEq:mEq)
4
3
2
1
0
(6)
(6)
(12)
(8)
(8)
(16)
SHR WKY ALL
CHRONIC
SHR WKY ALL
ACUTE.

DISCUSSION

SUMMARY OF FINDINGS

The Renal Function Curves

Previous examinations of renal function in conscious rats have utilized long-term urine collections in a metabolic cage. This is the first report of a preparation in which renal function of conscious rats may be evaluated during acute maneuvers performed days to weeks after the rats have recovered from surgery. I have used the preparation to examine the effects of acute ganglionic blockade on the relation between arterial pressure and sodium excretion rate in SHR and WKY rats. There are three major findings.

The first of these findings is that the renal function curve of the hypertensive SHR rat after ganglionic blockade was to the right of the age-matched WKY curve. Although this conclusion is supported by statistical analysis, it is not dependent on statistics; the conclusion is unchanged if one examines the renal function curves for the various age groups with the technique of "ocular demonstration" which was used by Harvey (1628).

Second, the separation of the SHR and WKY renal function curves after ganglionic blockade was already apparent in 8 to 12 week old rats. This is early in the period of developing spontaneous hypertension and long before any recognizable pathologic changes can be detected in SHR rats.

A third feature is the quantitative value of the separation between age-matched SHR and WKY renal function curves. In spite of an age-related

increase in resting blood pressure, both in WKY rats and especially in SHR rats, I could not detect a significant age-related change in the position of the renal function curves after ganglionic denervation for either SHR or WKY strains. A significant age effect was observed, however, in the horizontal distances between the SHR and WKY renal function curves measured at the chronic sodium excretion rate both before and after ganglionic blockade (Table 10, p 170). Both at 14 to 19 weeks and at 20 to 32 weeks, the horizontal separation between the renal function curves after ganglionic blockade for SHR and WKY rats was greater than the separation of the curves at 8 to 12 weeks. Furthermore, the separation between the renal function curves after ganglionic blockade was identical to the difference in resting mean arterial pressure. Since SHR and WKY rats had identical chronic sodium excretion rates, it follows that the horizontal separation between chronic SHR and WKY renal function curves was identical to the observed difference between the resting mean arterial pressures of age-matched SHR and WKY rats. Thus, the horizontal separation between the renal function curves for age-matched SHR and WKY rats was unchanged by ganglionic blockade.

My data augment that literature which describes the renal excretory behavior of the SHR rat. Since there were no significant differences between the three renal function curves of the three age groups of SHR or of WKY rats, it is of interest to plot a single renal function curve for each strain which incorporates the data from all ages of rats. In Figure 59, the linear regression lines which represent these two renal function curves are plotted together with the results of reports by other workers. Refer to Table 9 (p 167) for an analytical description

of my two curves.

Gilmore (1964) examined the baroreceptor-mediated effects of a change in arterial pressure on the sodium excretion rate in dogs. Stellate ganglion stimulation produced a decrease in renal vascular resistance and a natriuresis. Carotid artery occlusion produced an increase in renal vascular resistance which would be an antinatriuretic effect. Since the increase in renal vascular resistance after carotid artery occlusion was abolished by phenoxybenzamine, he surmised that the sympathetic nervous system mediates the changes in renal function which are brought about by an increase or a decrease in baroreceptor activity.

Other mechanisms could be described which also act on the kidney through the sympathetic nervous system to decrease the sodium excretion rate when arterial pressure decreases and to increase the sodium excretion rate when arterial pressure increase. With ganglionic blockade, these mechanisms will not operate and the renal function curve will be less steep than it would be without ganglionic blockade. It is to be expected, then, that the slope of the renal function curves after pharmacologic ganglionic blockade which I report are less steep than the curves which were measured by Enobakhare et al. (1978) and by Beierwaltes et al. (1978) in conscious rats without ganglionic blockade.

The renal function curves which I obtained are, however, much steeper than the curves reported by Tobian, Johnson, Lange et al. (1975) for isolated SHR and WKY kidneys. Can we account for the differences between my data and Tobian's data?

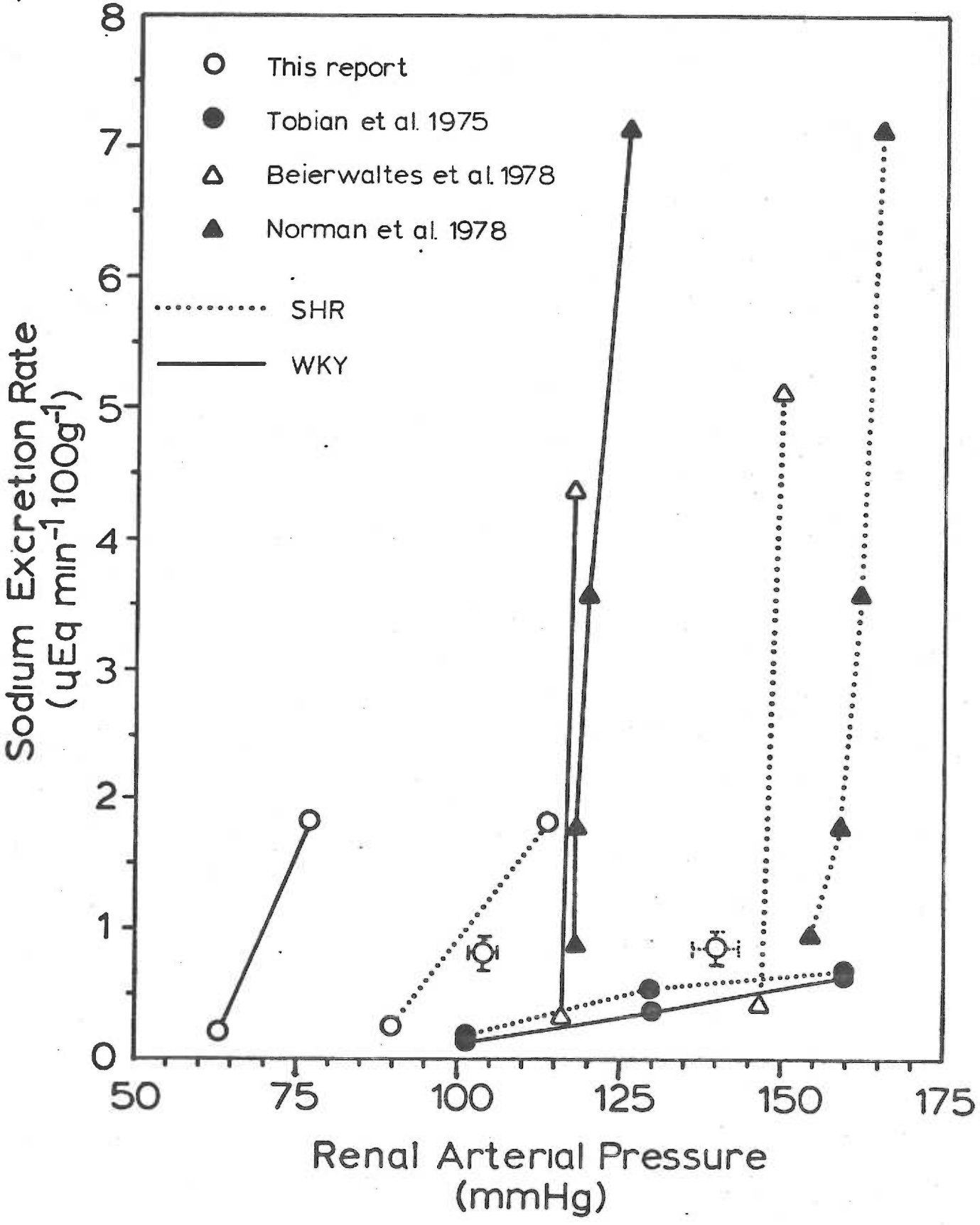
Using the in vivo data for cardiac output (Frohlich and Pfeffer,

1976) and renal blood flow as a percentage of cardiac output (Nishiyama, Nishiyama and Frohlich, 1976) presented in Table 3 (p 35) and the data on kidney weight and resting mean arterial pressure from this report, I have calculated that the in vivo renal vascular resistance is 15.9 and 13.6 $mmHg \cdot ml^{-1} \cdot min \cdot gKW^{-1}$ for SHR and WKY rats. Tobian reported resistances of 76.5 and 50.0 $mmHg \cdot ml^{-1} \cdot min \cdot gKW^{-1}$ for isolated blood-perfused kidneys of 17 week old SHR and WKY rats, respectively. Thus, his observed resistance values for isolated kidneys were five times greater than the in vivo resistance of SHR kidneys and four times greater than the in vivo renal resistance of WKY kidneys. I do not know if the low sodium excretion rate reported by Tobian was due to changes in intrarenal blood flow distribution or decreased GFR but it is clear that, under his experimental conditions, the kidneys were not behaving in a normal manner.

We may use some of the data from 24-hour urine collections to gain information about the chronic renal function curves. In Figure 59 the mean sodium excretion rates from 24-hour urine collections from SHR and WKY rats are plotted together with the mean arterial pressures recorded at the end of the 24-hour collection periods. If we assume that these points lie on the chronic renal function curves for these animals, it is clear that the SHR curve is to the right of the WKY curve. This analysis is not strictly correct, however, since the arterial pressure and the sodium excretion rate were not related to the same time period. I measured the sodium excretion rate from 24-hour urine collection. All arterial pressures in the conscious state were measured in the afternoon or early evening, a period during which the circadian pattern of blood

pressure in the rat is at its nadir (Bartter, 1977). Consequently, my arterial pressure measurements probably underestimate the average pressure over the 24 hour period of urine collections. This may explain why the chronic renal function curves measured by Norman, Enobakhare, DeClue et al. (1978) and by Beierwaltes and Arendshorst (1978) are both to the right of the points representing the chronic mean arterial pressure and sodium excretion rate from my data.

Figure 59: Comparison of renal function curves from several sources. Tobian, Johnson, Lange et al. (1975) used isolated blood-perfused kidneys from SHR and WKY rats. Beierwaltes and Arendshorst (1978) measured renal function curves in SHR and WKY rats 90 minutes after ether anesthesia and catheter implantation. They used acute saline loading to define two points on the renal function curve. Norman, Enobakhare, DeClue et al. (1978) used chronic saline loading in conscious rats over several days. Data from this report includes the renal function curves after ganglionic blockade (open circles connected by solid or dotted lines) and mean data for chronic mean arterial pressures and chronic sodium excretion rates from WKY rats (open circle with a solid cross representing the SEM) and from SHR rats (open circle with a dotted cross).


This report
Tobian et al. 1975
Beierwaltes et al. 1978
Norman et al. 1978
SHR
WKY
Sodium Excretion Rate
(uEq min-1 100g-1)
0
1
2
3
4
5
6
7
8
50
75
100
125
150
175
Renal Arterial Pressure
(mmHg)

Fall in Blood Pressure after Hexamethonium

There was a difference in the hypotensive response to hexamethonium between young SHR and WKY rats. In 8 to 12 week old SHR rats, mean arterial blood pressure 10 minutes after the infusion of 2 mg/100g hexamethonium was 78% of the resting blood pressure (Figure 32, p 137). In all other groups of SHR or of WKY rats, the blood pressure fell to between 62% and 67% of the resting value. This result does not agree with two other reports of the hypotensive effects of hexamethonium in the SHR rat. Table 12 summarizes these reports and compares them with the data for rats of similar age from my work.

Two groups (Numao, Suga and Iriuchijima, 1975; Judy, Watanabe, Henry et al., 1976) have reported that, after hexamethonium, blood pressure fell relatively more in the SHR rats than in an age-matched Wistar control group. Much of the difference between my data and the previous results may be explained on the basis of differences in methods. The other workers used Wistar, rather than WKY rats for controls and worked with either anesthetized rats or rats which were studied a few hours after surgery with pentobarbital anesthesia. Consequently, my finding that young SHR rats show significantly less hypotension with ganglionic blockade than do young WKY rats does not necessarily disagree with the other reports.

Table 12: The effect of hexamethonium on mean arterial pressure. Blood pressure was measured 10 minutes after the injection of a bolus of hexamethonium for this report. The time between hexamethonium infusion and pressure measurement is not reported for the other reports.

			Mean Arterial Pressure (mmHg)					
			SHR			Control		
Age (wks)	Control Rat	Conditions (Dose of C-6 mg/100g)	Basal	After C-6	C-6 / Basal	Basal	After C-6	C-6 / Basal
17-22	Wistar[1]	Conscious 3 hours after surgery with pentobarbital (3.0)	162	119	0.73	131	106	0.81
16-20	Wistar[2]	Pentobarbital 162 anesthesia (2.0)	162	65	0.40	106	57	0.54
14-19	WKY[3]	Conscious (2.0)	134	89	0.67	106	67	0.63

[1]Numao, Suga and Iriuchijima, 1975.

[2]Judy, Watanabe, Henry et al., 1976.

[3]This report.

CRITIQUE OF METHODS

If I am to generalize from my observations in these experiments to draw conclusions concerning the pathogenesis of hypertension in the SHR, I must show that the experimental procedures did not change significant characteristics of the animals. Did my experimental procedures alter any relevant characteristics of the rats?

Health of the Rats

The general health of the rats after surgery was determined using such features as the appearance of fur, their response to alerting sounds and their motor activity. Although my assessment of a rat's health was subjective, rats which I judged to be sick would usually die within two days. All rats were judged to be healthy at the time they were used to measure points for renal function curves.

There was a relative and an absolute weight loss in rats after surgery. This was true whether one catheter (Figure 34, p. 140) or three catheters (Figure 35, p.141) were implanted. But since there was no difference between SHR and WKY rats in the magnitude of the weight loss, it is unlikely that the weight loss had a major influence on the observed differences in the positions of SHR and WKY renal function curves.

An indication that the behavior of the kidneys of the experimental rats was not changed by surgery is the observation that the urinary volume output measured more than several days after surgery was similar to the rate of water intake that others have measured for SHR and WKY

rats. McConnell and Henkin (1973) found that SHR and WKY rats drank 9.1 ± 1.9 (SEM) and 10.8 ± 1.3 microliters·min^{-1}·100g^{-1}. Figure 36, (p.144) shows that after surgery the urinary volume output was quite variable but it was not significantly different than the water intake rate which was reported by others using unmodified rats.

I have shown that both plasma creatinine concentrations and creatinine clearances were normal on experimental days. Because I used small samples to measure creatinine concentration, these parameters had relatively large variances. Consequently, if there were any differences in the plasma creatinine concentration or creatinine clearance between SHR and WKY rats, they were not statistically apparent.

Acute and Chronic Conditions

Because of the finite transit time of fluid from Bowman's capsule to the bladder, how can I be sure that during acute experiments I was measuring the sodium excretion rate under conditions of ganglionic blockade? In other words, did I make my measurements under steady state conditions? Is it a coincidence that the mean value and variance of the acute and chronic sodium excretion rates are similar?

If, in acute urine collections, I was really measuring the chronic sodium excretion rate, I would not expect to see any correlation between the changes in mean arterial pressure and sodium excretion rate from the first to the second collection periods in those cases for which I was able to record for two successive 10 minute collection periods on the same day. I have shown that there is a positive correlation between the change in blood pressure and the change in sodium excretion rate in these cases (Figure 57, p 180).

The observation that the Na:K ratio of urine from acute experimental collections periods was 3.2 times the ratio of urine from 24-hour collections (Figure 58, p. 182) is additional evidence that urine collected under acute conditions differed from that collected under chronic conditions.

Using anesthetized rats, Ackermann (1978) collected bladder urine with a PE 50 tube. He measured the changes in urine volume, sodium excretion rate and potassium excretion rate which accompanied the infusion of the contents of a reservoir (containing an estimated 33% of the rats' blood volume) which had been equilibrated with the blood of

the rats. All excretion rates increased at 5 minutes and continued to increase during the 17 minute infusion period. I have already described (p.104) stop-flow experiments in rat kidneys (Harvey and Malvin, 1966) in which it took only three minutes for fluid to flow from the proximal tubules to the bladder.

Consequently, I conclude, both from my own observations and from several reports in the literature, that my use of urine collections periods which began four minutes after acute ganglionic blockade with or without a change in blood volume was appropriate to study the sodium excretion rate of the kidney under the experimental conditions.

The similarity between the chronic sodium excretion rate without ganglionic blockade and the sodium excretion rate after ganglionic blockade remain to be explained.

Let us examine the response to two relevant effects of hexamethonium infusion, namely systemic hypotension and pharmacologic blockade of the renal nerves. Decreased sodium excretion rate and subsequent sodium retention are well-known responses to hypotension. It has recently been suggested that renal denervation may cause natriuresis. Acute unilateral surgical renal denervation in the anesthetized rat was associated with a marked increase in sodium excretion rate without any change in the glomerular filtration rate of the denervated kidney (Colindres and Gottschalk, 1978). Mild stimulation of the renal nerves in the dog caused the sodium excretion rate to decrease in the absence of changes in glomerular filtration rate and renal blood flow. The antinatriuretic effect of renal nerve stimulation was not altered by inhibition of prostaglandin synthesis or by a competitive angiotensin II

antagonist (DiBona, Zambriski, Aguilera et al., 1977).

Judy, Watanabe, Henry et al. (1976), using anesthetized SHR and WKY rats, recorded renal nerve activity which was suppressed with the increased arterial pressure which accompanied norepinephrine influsion. Judy's report and the work of Colindres and Gottschalk (1978) indicate that, at least in the anesthetized rat, the sympathetic nervous system has a tonic influence on the kidney. The hypotensive, and therefore antinatriuretic, effect of hexamethonium influsion may have been offset in my study by a natriuretic effect of pharmacologic ganglionic blockade with hexamethonium. The effects of simultaneous hypotension and ganglionic blockade may explain the similarity between acute and chronic sodium excretion rates in my experiments.

Blockade of the Sympathetic Nervous System

I have shown (Figure 30, p.135) that, in the dose used in these experiments, hexamethonium effectively blocked vagal influences on the heart. I have assumed that I could generalize from this observation to conclude that hexamethonium will also produce ganglionic blockade of the kidney. There are several reasons that make this a reasonable assumption. Hexamethonium has been shown to be a highly specific ganglionic blocker in the rat; it will block ganglionic transmission at 1/1000th of the dose required to block post-ganglionic neural transmission (Quilliam and Shand, 1964). In addition, hexamethonium is effective in blocking autonomic influences on a variety of tissues (Wien and Mason, 1951) and the dose-response curves are similar for ganglia which influence either sympathetic or parasympathetic post-ganglionic nerves (Alonso de Forida, Cato, Ramirez et al., 1960).

In my analysis of the results of these experiments, I have also assumed that the effects of renal ganglionic blockade are due to blockade of the sympathetic nervous system. Some renal cortical nerve terminals, however, possess acetylcholinesterase activity or have clear vesicles of the type seen in cholinergic nerve terminals (Barajas, Wang and DeSantis, 1976). Barajas (1978) showed that all nerve terminals in the renal cortex also have small dense-cored vesicles of the type seen in adrenergic terminals and that administration of 6-OH dopamine caused all the nerves in the vicinity of the glomerular arterioles to undergo degeneration. Thus, whereas the enzymes required for cholinergic transmission are present in the renal

cortex, they may be associated only with adrenergic terminals. This would agree with the hypothesis of Burn and Rand (1965) that acetylcholine may be involved in adrenergic transmission. Although the role of the parasympathetic nervous system or cholinergic nerve terminals in the regulation of renal function is not well understood, for the purpose of this analysis I will assume that all the renal effects of ganglionic blockade can be explained on the basis of the blockade of the sympathetic nervous sytem.

COMPARISON OF OBSERVATIONS AND PREDICTIONS

Positions of the Renal Function Curves

My work is designed to investigate the hypothesis that the kidneys of prehypertensive SHR rats are normal but that the renal vasculature of the SHR will hypertrophy as hypertension develops so that, both with and without renal innervation, the renal function curve of the mature SHR will be shifted to the right of the WKY curve.

Based on this hypothesis, I have made three predictions concerning the age-matched renal function curves of SHR and WKY rats after ganglionic blockade:

1. As hypertension is beginning to become apparent in the SHR, the renal function curves of SHR and WKY rats after ganglionic blockade will not be separated significantly.
2. After hypertension is apparent in the SHR rats, the position of the renal function curve after ganglionic blockade of the SHR rat will be to the right of and parallel to the age-matched WKY curve.
3. As the severity of hypertension in SHR rats increases, the position of the renal function curve after ganglionic blockade of the SHR rat will shift further and further to the right of the WKY curve.

Have these predictions been supported by my observations?

I was not able to test the first prediction since I was not able to perform successfully the necessary surgery on SHR rats which were prehypertensive. Other reports indicate that, to examine prehyperten-

sive SHR rats, I would have to use rats younger than about 3 weeks (Lais, Rios, Boutelle et al., 1977). Such rats weigh less than 50 g. The experimental preparation which I used in this study can be used only with rats which weigh at least 100 g.

Since SHR rats were hypertensive at all ages which I studied, all of the renal function curves are relevant to the second prediction. The SHR renal function curve after ganglionic blockade was, for each age group, to the right of the age-matched WKY curve. Therefore, my observations support, in part, the second prediction. Furthermore, renal sympathetic nerve activity in the intact hypertensive SHR rat does not appear to be solely responsible for the rightward shift of the renal function curve in the undisturbed rat. After ganglionic blockade, there was no significant difference in the slopes of SHR and WKY renal function curves but there was a large variance in the observations used to define the slope. For this reason I cannot confidently conclude that the SHR and WKY renal function curves are parallel, though my data are consistent with this prediction.

Finally, I found that, after ganglionic blockade, the position of the SHR renal function curve apparently did not move further to the right as the SHR rats became increasingly hypertensive. I did, however, find that the separation between SHR and WKY renal function curves for the two older groups of rats was greater than the separation for the youngest group. Furthermore, the separation between SHR and WKY renal function curves after ganglionic blockade was identical to the difference in the resting blood pressures at the same age. Thus, blockade of

the autonomic nervous system did not modify the separation of the renal function curves. From this evidence, it appears that renal sympathetic nerve activity is not essential for the rightward shift of the renal function curve of hypertensive SHR rats. From this it follows that sympathetic nerve activity is probably not a major factor in the maintenance of hypertension in the mature SHR rat.

Sympathetic Nervous System Activity in Young SHR Rats

An important part of the hypothesis which I have proposed to explain the pathogenesis of hypertension in the SHR rat is that, in young SHR rats, the response of the sympathetic nervous system to alerting stimuli is greater than the response of age-matched WKY rats. Although I did not design this study to examine the activity of the sympathetic nervous system in young SHR rats, I did make an observation that is related to this question. If the sympathetic nervous system was more active in young SHR rats than in young WKY rats, I would expect that hexamethonium would have a greater hypotensive effect in young SHR rats than in WKY rats. I found, however, that the youngest group of SHR rats, from 8 to 12 weeks old, had the least percentage fall in blood pressure of the three groups (Figure 32, p 137). The reports that plasma norepinephrine and dopamine beta hydroxylase levels are elevated in 4 week old SHR rats (Grobecker, Roizen, Weise et al., 1975) and that recorded renal nerve activity is elevated in 16 week old SHR rats (Judy, Watanabe, Henry et al., 1976) appear to conflict with my observations. There are several explanations for this apparent conflict.

Circulating levels of adrenergic transmitters and enzymes are indirect indices of sympathetic activity. Also, Judy et al. (1976) recorded renal nerve activity in rats under pentobarbital anesthesia. If, as I hypothesize, the autonomic response to central stimulation is different for SHR rats, it is reasonable to believe that pentobarbital will differentially alter autonomic nerve activity in SHR and WKY rats.

Neither of the previous studies examined 8 to 12 week old rats.

The observation that there is a decreased dependence of peripheral vascular resistance on autonomic influences in 8 to 12 week old SHR rats must be considered in relation to the conclusion from this study that the role of the renal sympathetic nerve activity in determining the position of the renal function curve is similar in SHR and WKY rats. My observations do not rule out the possibility that sympathetic influences on the peripheral vasculature could be relatively lower in the young SHR rat than in the young WKY rat while renal nerve activity is normal.

Finally, my initial hypothesis predicts that enhanced sympathetic nervous system activity will be present only in the prehypertensive rat (i.e. the very young SHR rat) during periods of environmental stress. This study did not examine SHR rats at 3 weeks, an age at which the literature indicates that hypertension is first present. This may be the age at which SHR rats would be most likely to show enhanced sympathetic nervous system activity. It is possible that the 8 to 12 week old SHR rat which is already hypertensive is relatively volume-expanded and that the apparently lower sympathetic nervous system activity is a normal response to the volume expansion.

EVALUATION OF HYPOTHESES

On the Mechanisms of Spontaneous Hypertension

In Table 2 (p. 29) I presented a list of mechanisms which are capable of moving the position of the renal function curve of rats to the right and, therefore, may contribute to hypertension. With reference to the list, I will point out how the results of my studies contribute to an understanding of the etiology of spontaneous hypertension.

First, I will discuss those factors which I have examined in some detail. The position of the SHR renal function curve after ganglionic blockade was significantly to the right of the WKY curve. Ganglionic blockade with hexamethonium in acute experiments eliminated all sympathetic influences on the kidney since hexamethonium will not only directly block renal sympathetic nerves but will also block the release of catecholamines by the adrenal medulla (Hochman and Perlman, 1976). Therefore, this report does not support the proposal of Tobian, Johnson, Lange et al. (1975) that the autonomic nervous system plays a critical role in maintaining the SHR renal function curve to the right of the WKY curve. In fact, if subsequent work supports my observation that the right-left separation of SHR and WKY renal function curves after ganglionic blockade is the same as the difference in resting mean arterial pressure, we may conclude that the sympathetic nervous system is not a significant factor in maintaining the rightward position of the chronic SHR renal function curve. While differences in sympathetic nervous system activity or reactivity between SHR and WKY rate may be responsible for initiating spontaneous hypertension, the sympathetic

nervous system does not appear to be required for the maintenance of hypertension in SHR rats.

If PRA is a valid index of renin-angiotensin system activity, this work provides no evidence that this system is important in the maintenance of spontaneous hypertension since there was no difference between SHR and WKY PRA under basal conditions or 25 minutes after i.v. hexamethonium. Normal PRA in the SHR does not, however, necessarily mean that the renin-angiotensin system is unimportant in the maintenance of spontaneous hypertension. SHR renal vasculature may, for instance, have enhanced reactivity to angiotensin II.

It takes 1.5 hours for changes in exogenous aldosterone concentration to influence sodium transport rate in isolated rat renal tubules (Castles and Williamson, 1967). Thus, any differences in circulating mineralocorticoid concentration between SHR and WKY rats which were present under chronic conditions should also be present during acute experiments. Since there was no difference between the urinary Na:K ratio in SHR and WKY rats either under chronic conditions or during acute experiments, my results are not consistent with the proposition that increased circulating mineralcorticoids contribute to hypertension in the SHR.

Loss of renal mass is unlikely to be an important contributor to the rightward position of the SHR renal function curve since kidney weight, relative to body weight, was identical in SHR and WKY rats (Figure 56, p.176).

The observations that the SHR renal function curve is to the right of the WKY curve even after ganglionic blockade is consistent with the hypothesis that, in the mature SHR, there is a structurally-based increase in renal vascular resistance which may be responsible for the rightward position of the renal function curve both in the intact SHR and after pharmacologic ganglionic blockade. With arteriolar hypertrophy in the SHR kidney, an increased perfusion pressure may be required for normal levels of renal blood flow, glomerular filtration rate and sodium excretion rate. If the renal arteriolar hypertrophy causes an increased renal vascular resistance that is not dependent on elevated sympathetic nerve activity in the SHR, the vascular hypertrophy could account for the rightward shift of the renal function curve in mature SHR rats both with and without ganglionic blockade.

Data from both stop-flow experiments (Gothberg, Hallback, Lundin, et al., 1976) and from single-nephron studies (Azar, Johnson and Tobian, 1976) in the SHR indicate that both afferent and efferent arteriolar resistances are increased but the ratio of afferent to efferent resistance is higher in SHR rats than in normotensive Wistar rats. Thus, renal vascular hypertrophy may act to maintain hypertension in the SHR by requiring a hypertensive perfusion pressure for a normal glomerular capillary pressure.

Since I did not study or control for plasma colloid osmotic pressure, vasopressin, prostaglandins, plasma calcium concentrations or natriuretic hormone in my experiments, this report provides no data on the role of these factors in maintaining the rightward position of

the SHR renal function curve relative to the WKY curve.

One cannot hope to reveal the major cause or causes of the rightward position of the SHR renal function curve from a single experimental protocol. The role of each of the factors listed in Table 2 (p. 29) in the genesis or maintenance of spontaneous hypertension must be rigorously tested. By excluding individual factors with well-controlled experiments using physiological preparations, we may shorten the list of hypertensinogenic candidates to those factors which have resisted all our efforts to discredit their role in spontaneous hypertension. The remaining factors could then be considered the most likely causes of hypertension in SHR rats. Of course, we can only study those factors which we have defined. There will always remain the possibility that an unexpected factor is important in the pathogenesis of hypertension in the SHR rat.

Modifications to the Initial Hypothesis

How do these experiments change my original hypothesis? On the basis of my data, there is no reason to change my belief that, in the mature SHR, a structurally-based increase in renal vascular resistance is responsible for the rightward position of the chronic renal function curve relative to the WKY curve. Renal vascular hypertrophy may be considered to be an adaptive response to hypertension, allowing for normal renal blood flow, glomerular filtration rate and sodium excretion rate at the increased perfusion pressure. Renal vascular hypertrophy is, however, maladaptive for the maintenance of normotension. A normal rat kidney has an elevated sodium excretion rate and a decreased body fluid volume when perfusion pressure is hypertensive. Therefore, I may still assert that, because renal vascular hypertrophy can keep the SHR renal function curve to the right of the WKY curve, it may be responsible for maintaining hypertension in the mature SHR rat after the onset of spontaneous hypertension.

This work did not, however, provide any support for the notion that there is increased sympathetic nervous system activity in 8 to 12 week old SHR rats. Sympathetic activity has not, however, been ruled out as a factor in the initiation of hypertension. We are forced, however, to consider additional mechanisms as initiators of hypertension. On the basis of other reports, increased sympathetic nerve activity, increased circulating adrenal catecholamines, increased vascular reactivity to pressor agents such as catecholamines and angiotensin II, altered local or circulating prostaglandins and

genetically-determined renal vascular hypertrophy are among the more prominent candidates to be initiators of hypertension in the young SHR rat. Because of Folkow's report of a normal perfusion pressure-GFR relation in isolated kidneys from 5 week old SHR rats (Folkow, Gothberg, Lundin et al., 1977a), I would not propose that genetically-determined renal vascular hypertrophy initiates hypertension in the SHR.

Thus, although the mechanism or mechanisms which are responsible for the genesis of hypertension in the SHR remain speculative, my data provide support for the hypothesis that, in a favorable genetic setting, hypertension can cause renal vascular hypertrophy which, in turn, contributes to the maintenance of hypertension by keeping the chronic renal function curve of the SHR rat to the right of the WKY curve. It is important to recognize that, whereas I propose that there is a genetic predisposition for rapid progression of renal vascular hypertrophy in the SHR, I also believe that vascular hypertrophy will not occur in the absence of some non-renal initiator of hypertension.

Lundgren, Hallback, Weiss et al., (1974) have shown that, in Wistar rats with renal hypertension, functional vascular hypertrophy is complete in 1 to 2 weeks. Therefore, vascular hypertrophy occurs so rapidly that it would be difficult to observe a definitive time lag between the onset of hypertension and the development of renal vascular hypertrophy.

Future Work

Because the techniques which I used in this work allow one to examine renal function in conscious, unrestrained rats, they are obviously of use in a wide range of pharmacological and physiological studies on rats which are at least 8 to 12 weeks old. The methods can, of course, be used in rats other than SHR or WKY rats. Some of the more obvious studies which would add to our knowledge of the SHR rat include the effects of long-term angiotensin antagonists and converting enzyme inhibitors on renal function, the assessment of exaggerated natriuresis in the SHR rat and more detailed studies of renal tubular function using stop-flow methods.

My proposals for future work are guided, however, by the belief that our understanding of the initiation of hypertension in the SHR rat is rudimentary compared with the progress that has been made over the past fifteen years in defining the physiology of the hypertensive SHR rat. We may study the renal function curve in pre-hypertensive SHR rats either by using antihypertensive medications to postpone the onset of hypertension until the rats are large enough to use the techniques which are reported in this work or by studying rats younger than 3 weeks old.

It would be rather easy to use my techniques on rats at least 8 to 12 weeks old which have been treated with antihypertensive medications which have been shown to be effective in preventing hypertension for up to 60 weeks in SHR rats (Freis and Ragan, 1975). Alternatively, I could develop new techniques for catheterization which could be used

in rats 3 weeks old when SHR rats are not hypertensive but weigh only 35 grams. The major obstacle which I foresee would be to implant an arterial catheter that was so small that it would not reduce aortic blood flow. It may be possible to do this with a PE 8 tube which had been drawn out to a smaller diameter in a stream of hot air. Placement of this catheter in the femoral artery, while it may increase the risk of thrombosis at the catheter tip, would minimize aortic occlusion which was a problem in the present study when PE 8 tubing was placed in the abdominal aortas of 100g rats.

SUMMARY AND CONCLUSIONS

I report a method which can be used to measure the short-term renal excretory and arterial pressure responses to acute pharmacologic interventions in conscious unrestrained rats. I used this method to examine the role of the sympathetic nervous system in the maintenance of the chronic rightward shift of the renal function curve in the spontaneously hypertensive (SHR) rat relative to the normotensive Wistar-Kyoto (WKY) rat.

Measurements of chronic sodium excretion rate and mean arterial pressure in this work agree with other reports that there is a rightward shift of the chronic renal function curve in hypertensive SHR rats. I also found that the renal function curves in SHR rats which were 8 to 12, 14 to 19 or 20 to 32 weeks old were to the right of the curves for age-matched WKY rats even after ganglionic blockade with hexamethonium. SHR rats were hypertensive relative to WKY rats for all ages and the blood pressure of SHR rats increased with age.

After ganglionic blockade, the separation between SHR and WKY renal function curves for each of the three age groups was similar to the difference in resting mean arterial pressure between SHR and WKY rats at the same age. Thus, ganglionic blockade had no effect on the separation between SHR and WKY renal function curves.

The persistence of the rightward shift of the SHR renal function curve after ganglionic blockade indicates that the sympathetic nervous system is not required for the maintenance of the rightward shift of

the chronic renal function curve in hypertensive SHR rats. Since I argue that the rightward shift of the renal function curve is necessary for the maintenance of hypertension, these data suggest that the sympathetic nervous system is not solely responsible for hypertension in the SHR rat.

These observations are consistent with the alternative hypothesis that structural changes in the renal vasculature are responsible for the rightward shift of the SHR renal function curve and may, therefore, be crucial for the maintenance of hypertension in the mature SHR rat.

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Appendix : Tabulation of data pairs of the mean arterial pressure (MAP - mmHg) and the sodium excretion rate ($U_{Na}V$ - microEq·min^{-1}·100g BW^{-1}).

Rat Number	Data Pairs (MAP, $U_{Na}V$)					Mean of the Data Pairs for the Rat
8-12 week old SHR Rats						
78-38	99.8, 0.707	83.1, 0.286				91.4, 0.497
78-41	95.9, 0.635	84.6, 0.640	85.2, 1.271	102.8, 1.079		92.1, 0.906
78-52	103.0, 1.90					103.0, 1.90
78-70	88.8, 0.899					88.8, 0.899
78-80	78.9, 0.356					78.9, 0.356
78-81	91.3, 0.586	93.0, 0.369	61.5, 0.607			81.9, 0.521
78-88	102.5, 0.625 103.9, 0.748	85.6, 1.402	94.1, 1.112	107.8, 1.155	95.1, 0.976	98.1, 1.003
78-89	93.5, 0.375 113.7, 0.461	103.3, 0.368 109.3, 1.536	107.5, 0.333	120.3, 1.520	110.2, 1.448	108.3, 0.863
78-91	104.7, 0.702 98.3, 1.835	103.1, 0.708 111.0, 0.586	112.3, 1.603	109.6, 0.956	98.9, 1.495	105.4, 1.126
14-19 week old SHR rats						
78-24	80.5, 0.602	87.0, 0.340				83.7, 0.471
78-27	91.7, 0.854					91.7, 0.854
78-107	111.0, 0.676					111.0, 0.676
78-112	76.2, 0.262	75.9, 0.796	53.9, 0.144	74.6, 0.706	85.8, 0.807	73.2, 0.543
78-115	106.7, 0.766	90.1, 0.570	93.2, 0.548	83.2, 0.547		93.3, 0.608

Appendix (continued)

Rat Number	Data Pairs (MAP, $U_{Na}V$)					Mean of the Data Pairs for the Rat
14-19 week old SHR rats (continued)						
78-118	98.1, 1.00	91.0, 0.971	71.8, 0.433	65.0, 0.312	90.0, 0.503	83.2, 0.644
78-120	108.9, 0.555 102.2, 0.618	92.3, 0.264 114.5, 0.865	84.6, 0.387	100.6, 0.365	106.8, 0.568	100.4, 0.593
20-32 week old SHR rats						
78-129	105.0, 0.360					105.0, 0.360
78-12	125.0, 0.220 106.8, 0.369	131.0, 1.29	109.0, 0.55	94.7, 0.13	137.7, 0.49	117.4, 0.508
78-17	83.0, 0.117	132.0, 0.732				107.5, 0.425
78-71	81.0, 0.319	86.1, 0.207				83.6, 0.263
78-92	80.4, 0.335 154.0, 1.269	111.2, 1.021	102.8, 0.849	106.0, 1.299	135.4, 1.721	114.9, 1.082
78-102	84.4, 0.446					84.4, 0.446
78-132	95.6, 0.190 123.6, 0.798	75.9, 0.374	111.2, 0.337	101.0, 0.483	112.6, 0.942	103.3, 0.521
78-136	75.2, 0.610 90.0, 0.881 93.8, 0.548	62.2, 0.563 83.3, 0.601 97.3, 0.545	77.7, 1.123 84.5, 0.694 104.0, 0.573	75.3, 0.565 93.8, 1.022	88.7, 0.823 83.3, 0.406	85.4, 0.689

Appendix (continued)

Rat Number	Data Pairs (MAP, $U_{Na}V$)					Mean of the Data Pairs for the Rat
8-12 week old WKY rats						
78-47	68.6, 0.71	67.1, 0.58	71.2, 0.97			69.0, 0.753
78-44	75.2, 0.406	78.6, 1.240				76.9, 0.823
78-73	91.6, 1.61					91.6, 1.61
78-78	64.2, 0.943	73.4, 1.072	69.4, 0.400	79.0, 0.849		71.5, 0.816
78-84	85.0, 0.653					85.0, 0.653
78-141	63.6, 0.752	54.0, 0.729	62.4, 0.511	80.7, 0.792		65.0, 0.706
14-19 week old WKY rats						
78-110	54.9, 0.765 55.6, 0.524	63.1, 0.758 49.1, 1.160	67.6, 1.411 46.3, 0.743	59.1, 0.814	58.6, 0.969	56.8, 0.893
78-114	67.5, 0.966					67.5, 0.966
78-117	72.9, 0.593	54.6, 0.124				63.8, 0.359
78-122	71.0, 1.105 64.2, 0.662 79.6, 0.961	65.8, 0.794 65.0, 0.694	72.5, 0.665 70.2, 0.459	66.3, 0.652 69.1, 1.081	50.0, 0.255 77.3, 1,036	68.3, 0.760
78-134	63.6, 0.590 63.1, 1.498	60.3, 0.658	61.3, 0.724	54.0, 0.548	59.8, 0.602	60.4, 0.770

Appendix (continued)

Rat Number	Data Pairs (MAP, $U_{Na}V$)					Mean of the Data Pairs for the Rat
20-32 week old WKY rats						
78-110	54.9, 0.765 55.6, 0.524	63.1, 0.758 49.1, 1.160	67.6, 1.411 46.3, 0.742	59.1, 0.814	58.6, 0.969	56.8, 0.893
78-114	67.5, 0.966					67.5, 0.966
78-117	72.9, 0.593	54.6, 0.124				63.8, 0.359
78-122	71.0, 1.105 64.2, 0.662 79.6, 0.961	65.8, 0.794 65.0, 0.694	72.5, 0.665 70.2, 0.459	66.3, 0.652 69.1, 1.081	50.0, 0.255 77.3, 1.036	68.3, 0.760
78-134	63.6, 0.590 63.1, 1.498	60.3, 0.658	61.3, 0.724	54.0, 0.548	59.8, 0.602	60.4, 0.770