

INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

- 1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.**
- 2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.**
- 3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.**
- 4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.**
- 5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.**

University Microfilms International

300 North Zeeb Road

Ann Arbor, Michigan 48106 USA

St. John's Road, Tyler's Green

High Wycombe, Bucks, England HP10 8HR

77-8829

OHANIAN, Edward Vahram, 1944-
GENETIC INFLUENCE ON CADMIUM-
INDUCED HYPERTENSION.

City University of New York, Ph.D., 1977
Physiology

Xerox University Microfilms, Ann Arbor, Michigan 48106

© 1976

EDWARD VAHRAM OHANIAN

ALL RIGHTS RESERVED

GENETIC INFLUENCE ON CADMIUM-INDUCED HYPERTENSION

by

EDWARD V. OHANIAN

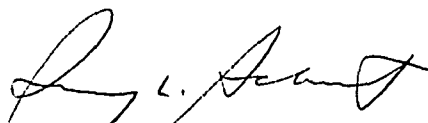
A dissertation submitted to the Graduate Faculty
in Biomedical Sciences in partial fulfillment of
the requirements for the degree of Doctor of
Philosophy, The City University of New York.

1976

This manuscript has been read and accepted for the Graduate Faculty
in Biomedical Sciences in satisfaction of the dissertation require-
ment for the degree of Doctor of Philosophy.

12/6/76

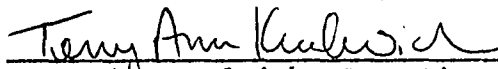
date



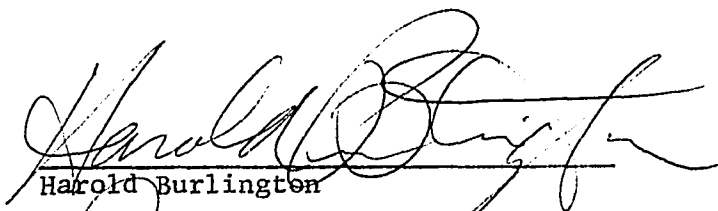
Irving L. Schwartz, Chairman of
Examining Committee

12/7/76

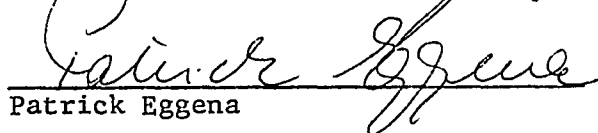
date



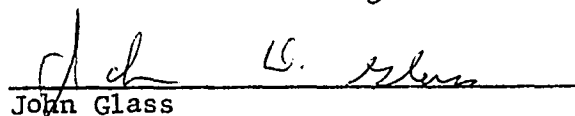
Terry Ann Krulwich, Executive Officer



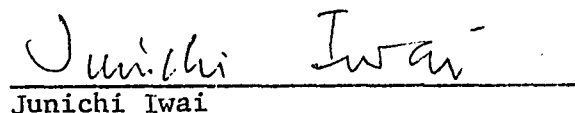
Harold Burlington



Patrick Eggena



John Glass



Junichi Iwai

The City University of New York

Abstract

GENETIC INFLUENCE ON CADMIUM-INDUCED HYPERTENSION

By

Edward V. Ohanian

Advisor: Professor Irving L. Schwartz

Schroeder suggested that at least part of human "essential" hypertension is associated with inapparent cadmium exposure. He based this hypothesis on two major premises: first, that hypertensive subjects had abnormally high accumulation of cadmium in the kidneys. Second, that chronic cadmium feeding induced hypertension in rats. However, the failure of various studies to confirm a clear relation between cadmium intake and hypertension have made Schroeder's hypothesis doubtful.

In the early sixties Dr. Lewis K. Dahl developed two unique strains of rats originally derived from a common pool of Sprague-Dawley ancestors by selective inbreeding. They are designated Dahl hypertension-resistant (R) and Dahl hypertension-sensitive (S) strains of rats because of their resistance and susceptibility to develop hypertension in response to chronic excess salt ingestion as well as to other experimental procedures. The development of hypertension in S rats required both the appropriate genetic substratum and a hypertensinogenic stimulus for full expression. Therefore, it appeared rewarding to design acute and chronic cadmium experiments to explore the genetic influence on the effects of cadmium on blood

pressure and other parameters in R and S rats.

The pressor response to cadmium (0.4 mg/kg, intra-arterially) in S rats was twice as high as in R rats. Cadmium (0.4 mg/kg, intra-arterially) did not sensitize the blood vessels of R or S rats to the action of intra-arterially administered angiotensin II (20 and 40 ng/kg) or norepinephrine (200 and 400 ng/kg).

When an injection of cadmium (2 mg/kg, intraperitoneally) was administered to weanling (3 weeks of age) male and female rats of either strain and followed by a second injection of cadmium at 6 weeks of age, female S rats developed hypertension. By contrast, the R rats and the male S rats showed no significant changes ($p > 0.05$) in blood pressure. Cadmium concentrations in renal and hepatic tissues were significantly higher ($p < 0.001$) in cadmium-injected male and female S rats than in cadmium-injected R rats. The kidneys of female S rats given cadmium showed the renal vascular changes characteristic of renal hypertension of moderate degree. Contrary to earlier findings in male S weanlings, identical doses of cadmium and an identical dosing interval produced hypertension in adult S males.

Cadmium (1 and 2 mg/kg, intraperitoneally) enhanced the development of salt-induced hypertension in weanling female S rats. Furthermore, the morbidity and mortality of two-kidney Goldblatt hypertension was considerably accelerated by cadmium (2 mg/kg, intraperitoneally) in adult S males. Conversely, cadmium had no influence on mild renal hypertension in the counterpart R rats.

Groups of female R and S rats were chronically fed 0 (Control), 1, 2.5, 5 and 10 mg cadmium/L in the drinking water and either low

(0.4% NaCl) or high (4% NaCl) salt diet from the time they were weaned until they were 31 weeks old. Cadmium (1 to 5 mg/L) produced hypertension in S rats on low salt diet. Cadmium (1 and 2.5 mg/L) accelerated the development of salt-induced hypertension in S rats. R rats remained normotensive irrespective of the cadmium feeding and diet throughout the study. Cadmium lowered plasma cholesterol levels in either strain on low salt diet. Marked proteinuria was detected in cadmium-fed S rats on low salt diet. Gross cardiac hypertrophy was observed in S rats fed the lowest dose of cadmium (1 mg/L) and low salt diet. Cadmium had no influence on susceptibility to infection, life span, body weight, tumor formation, and plasma renin activity in either strain on both diets. The concentrations of cadmium in the kidney and liver tissues of cadmium-fed S rats on either diet were significantly higher ($p < 0.001$) than that of cadmium-fed R rats. Mild to moderate nephrosclerosis was present in cadmium-fed rats (1 to 5 mg/L) on low salt diet.

The data cited in these studies indicate for the first time that the genetic substratum is a critical determinant of the development of experimental hypertension following exposure to cadmium.

DEDICATION

To the late Dr. Lewis K. Dahl (1914-1975) who first stimulated my interest in this field and gave constant support and encouragement over the years.

ACKNOWLEDGEMENTS

I am particularly grateful to the U. S. Energy Research and Development Administration and the Medical Research Center of Brookhaven National Laboratory for allowing me to use their facilities during the course of my research.

I am immensely indebted to my advisor, Dr. Irving L. Schwartz, for his invaluable advice and constant guidance throughout my graduate career. Through his consistent willingness to discuss and alleviate problems as they arise, Dr. Schwartz maintains a pleasurable and uniquely rewarding research environment.

Special thanks are owed to Dr. Junichi Iwai for his confidence in me, for his guidance and support through difficult times and for his advice concerning my experimental approaches.

I am deeply indebted to Drs. Patrick Eggena, Richard Friedman, John Glass and Gaston Schaechtelin for their continued advice, interest, and critical reading of this manuscript.

I would like to extend my appreciation to Drs. Jean Laissue and Francois Debelac who found the time to make the histological examinations.

I would like also to express my gratitude to Dr. Richard Friedman, Howard Pate and Keith Thompson for their invaluable assistance with the statistical analysis.

I wish also to acknowledge the excellent technical assistance of Frank Amendola, Bernice Armstrong, Francis Brown, Geraldine Callister, Katherine Conkling, George Leidl, David McChesney,

Juliet Mercer, Samuel Miller, Carl Otto, Lorraine Tassinari and Ralph Tuthill. I am also thankful to Martha Heine who taught me most of the methods used herein.

I would like to thank Marie Susa for generously donating her time to type this manuscript.

PUBLICATIONS

Publications resulting from this experimental work:

1. Ohanian, E. V., and L. K. Dahl. 1976. Acute effects of intra-arterial cadmium injection on blood pressure in hypertension-sensitive (S) and resistant (R) rats. Feder. Proc. 35: 555.
2. Ohanian, E. V., L. K. Dahl, and J. Iwai. 1976. Genetic influence on hypertension induced by cadmium in Dahl hypertension-resistant and hypertension-sensitive rats. In D. D. Hemphill (Ed.), Trace Substances in Environmental Health-X. Columbia, Missouri. (In press)

LIST OF ABBREVIATIONS

Angiotensin II	Angiotensin II
Blood pressure	Blood pressure
Blood urea nitrogen	Blood urea nitrogen
Body weight	Body weight
Cadmium	Cadmium
Intra-arterial (ly)	Intra-arterial (ly)
Intraperitoneal (ly)	Intraperitoneal (ly)
Norepinephrine	Norepinephrine
Sodium chloride	Sodium chloride
Plasma renin activity	Plasma renin activity
Radioimmunoassay	Radioimmunoassay
Dahl hypertension-resistant	Dahl hypertension-resistant
Dahl hypertension-sensitive	Dahl hypertension-sensitive
Standard error of the mean	Standard error of the mean

TABLE OF CONTENTS

	Page
I. INTRODUCTION	1
II. MATERIALS AND METHODS	15
A. The Rats and their General Care	15
B. The Rat Food and Drinking Water	15
C. Direct Blood Pressure Measurement	18
D. Indirect Blood Pressure Measurement	19
E. Body Weight Measurement	20
F. Determination of Blood Urea Nitrogen	20
G. Determination of Plasma Cholesterol	21
H. Determination of pH, Protein, Glucose and Blood in Urine	23
I. Determination of Plasma Renin Activity	25
J. Determination of Cadmium Concentrations in Tissues	29
K. Preparation of Tissues for Microscopic Examination	31
L. Statistical Analysis of the Data	31
III. EXPERIMENTAL DESIGN	32
A. Acute Cadmium Study	32
B. Chronic Cadmium Study 1	34
C. Chronic Cadmium Study 2	35
D. Chronic Cadmium Study 3	36
E. Chronic Cadmium Study 4	37
F. Chronic Cadmium Study 5	38
G. Chronic Cadmium Study 6	40
IV. RESULTS	43
A. ACUTE CADMIUM STUDY	43
1. Effect of Intra-Arterial Cadmium Injection on Blood Pressure	43
2. Acute Blood Pressure Responses to Pre and Post- Cadmium Injections of Angiotensin II	43
3. Acute Blood Pressure Responses to Pre and Post- Cadmium Injections of Norepinephrine	49

(Table of Contents continued)

	Page
B. CHRONIC CADMIUM STUDY 1	49
1. Effect of Cadmium Injection on Blood Pressure .	49
2. Effect of Cadmium Injection on Body Weight . .	56
3. Cadmium Concentrations in Tissues	61
4. Renal Vascular Changes in Response to Cadmium Injection	61
C. CHRONIC CADMIUM STUDY 2	65
1. Effects of Cadmium Injection on Blood Pressure and Body Weight	65
2. Cadmium Levels in the Renal Cortex and Medulla and Liver at the Onset of Cadmium-Induced Hypertension	67
D. CHRONIC CADMIUM STUDY 3	67
1. Effect of Cadmium Injection on Blood Pressure .	67
2. Effect of Cadmium Injection on Body Weight . .	74
3. Cadmium Concentrations in Tissues	76
4. Renal Vascular and Hepatic Changes in Response to Cadmium Injection	76
E. CHRONIC CADMIUM STUDY 4	79
1. Effect of Cadmium Injection on the Course of Salt-Induced Hypertension	79
2. Effect of Cadmium Injection on Body Weight . .	83
3. Renal Vascular Changes in Response to Cadmium Injection	83
F. CHRONIC CADMIUM STUDY 5	83
1. Effect of Cadmium Injection on the Course of Two-Kidney Goldblatt Hypertension	83
2. Effect of Cadmium Injection on Body Weight . .	88
3. Cadmium Concentrations in Tissues	90
4. Renal Vascular changes in Response to Cadmium Injection	90

(Table of Contents continued)

	Page
G. CHRONIC CADMIUM STUDY 6	93
1. Intake of Cadmium in Water	93
2. Effect of Cadmium Feeding on Blood Pressure . .	96
3. Effects of Cadmium Feeding on Body and Organ Weights	102
4. Effect of Cadmium Feeding on Tumor Formation .	104
5. Effects of Cadmium Feeding on Blood Urea Nitrogen and Plasma Cholesterol	104
6. Effect of Cadmium Feeding on Urinary pH, Glucose, Blood and Protein	107
7. Cadmium Concentrations in Tissues	109
8. Renal Vascular and Hepatic Changes in Response to Cadmium Feeding	109
9. Effect of Cadmium Feeding on Plasma Renin Activity	111
V. DISCUSSION	115
VI. BIBLIOGRAPHY	130

LIST OF TABLES

		Page
Table 1	Initial blood pressure and rise of blood pressure in response to intra-arterial control injection of 150 mM NaCl in female R and S rats.	44
Table 2	Renal vascular changes in female R and S rats injected with cadmium.	64
Table 3	Effects of cadmium injection on blood pressure and body weight in female R and S rats.	66
Table 4	Effect of cadmium injection on body weight in male and female R and S rats.	75
Table 5	Renal vascular and hepatic changes in adult male and female R and S rats injected with cadmium.	80
Table 6	Effect of cadmium injection on body weight in female S rats on high salt diet.	84
Table 7	Renal vascular changes in female S rats fed high salt diet and injected with cadmium.	85
Table 8	Effect of cadmium injection on body weight in male R and S rats with renal artery constriction.	89
Table 9	Renal vascular changes in cadmium-injected male R and S rats with renal hypertension.	94

(List of Tables continued)

		Page
Table 10	Cadmium intake of female R and S rats exposed to 1, 2.5, 5 and 10 mg cadmium/L drinking water.	95
Table 11	Effect of cadmium feeding on body weight in female R and S rats.	103
Table 12	Effect of cadmium feeding on organ weight in female R and S rats.	105
Table 13	Effects of cadmium feeding on blood urea nitrogen and plasma cholesterol in female R and S rats.	106
Table 14	Effect of cadmium feeding on urinary protein concentration in female R and S rats.	108
Table 15	Cadmium concentrations in the kidney and liver of female R and S rats fed cadmium for 28 weeks.	110
Table 16	Renal vascular changes in female R and S rats fed cadmium for 28 weeks.	112
Table 17	Plasma renin activity in female R and S rats fed cadmium for 28 weeks.	113

LIST OF FIGURES

	Page
Figure 1 Schematic representation of selective inbreeding from successive generations of rats.	12
Figure 2 Mean changes in blood pressure in response to intra-arterial cadmium injections in R and S rats.	46
Figure 3 Mean rise of blood pressure in response to pre and post-cadmium injections of angiotensin II.	48
Figure 4 Mean rise of blood pressure in response to pre and post-cadmium injections of norepinephrine.	51
Figure 5 Effect of cadmium injection on blood pressure in male R and S rats.	53
Figure 6 Effect of cadmium injection on blood pressure in female R and S rats.	55
Figure 7 Effect of cadmium injection on body weight in male R and S rats.	58
Figure 8 Effect of cadmium injection on body weight in female R and S rats.	60
Figure 9 Cadmium concentrations in the kidney and liver of R and S rats injected with cadmium.	63
Figure 10 Cadmium concentrations in the renal cortex and medulla and liver of female R and S rats at the onset of cadmium-induced hypertension.	69

(List of Figures continued)

	Page
Figure 11 Effect of cadmium injection on blood pressure in adult male R and S rats.	71
Figure 12 Effect of cadmium injection on blood pressure in adult female R and S rats.	73
Figure 13 Cadmium concentrations in the kidneys and liver of R and S rats exposed to cadmium by injection.	78
Figure 14 Effect of cadmium injection on salt-induced hypertension in female S rats.	82
Figure 15 Effect of cadmium injection on two-kidney Goldblatt hypertension in male R and S rats.	87
Figure 16 Cadmium concentrations in the kidneys and liver of male R and S rats with renal hypertension.	92
Figure 17 Effect of chronic cadmium feeding on blood pressure in female R and S rats on low salt diet.	98
Figure 18 Effect of chronic cadmium feeding on blood pressure in female R and S rats on high salt diet.	100

I. INTRODUCTION

Many mineral elements occur in living tissues in such small amounts that the early workers were unable to measure their precise concentrations with the analytical methods then available. For this reason they were often mentioned as occurring in "traces" and the term trace elements arose to describe them. This term has remained in popular usage despite the fact that virtually all the trace elements can now be estimated with great accuracy and precision.

A meaningful classification of the trace elements is difficult because the only characteristic that they have in common is their occurrence in the tissues of plants, animals, and microorganisms in low concentrations. The magnitude of these concentrations varies greatly among different elements and, for particular elements, among different living organisms and parts of such organisms. Such differences apply equally to those elements known to be nutritionally essential and those for which no vital function has yet been found. Thus the requirements of mammals for zinc and copper are many times greater than those for iodine and selenium, and the concentrations of iron and zinc in animal tissues are normally much greater than those of manganese and cobalt. Furthermore, some apparently non-essential elements such as bromine, cadmium and silicon frequently occur in the blood and tissues of the body at levels substantially higher than those of most of the known essential trace elements.

The trace elements can be divided conveniently into two groups:

the dietary essentials and the nonessentials. Certain trace elements are essential for the life or health of both animals and plants. Vanadium, chromium, manganese, iron, cobalt, copper, zinc, and molybdenum are metals having a definitive role in mammalian metabolism or having demonstrated effects on growth or survival. Selenium, fluorine and iodine are nonmetals with biologic effects. In addition, strontium may play a role in the formation of bones and teeth. So basic are the functions of many of these essential elements, that it would not be surprising to find alterations in their concentrations in a large number of conditions involving malfunction or destruction of tissue, with secondary changes in tissue components. Furthermore, because metallic cofactors of enzymes or activators of enzyme systems are essential for basic metabolic reactions, it would not be surprising to discover chronic diseases resulting from tissue deficiency of one or another trace element.

According to Cotzias (1967), a trace element can be dignified with the formal title "essential" if it meets the following criteria: (1) it is present in all healthy tissues of all living things; (2) its concentration from one animal to the next is fairly constant; (3) its withdrawal from the body induces reproducibly the same structural and physiological abnormalities regardless of the species studied; (4) its addition either prevents or reverses these abnormalities; (5) the abnormalities induced by deficiency are always accompanied by pertinent specific biochemical changes; and (6) these biochemical changes can be prevented or cured when the deficiency is prevented or cured.

Some 20 to 30 trace elements which do not meet these requirements occur more or less constantly in highly variable concentrations in living tissues. They include aluminum, antimony, mercury, cadmium, germanium, vanadium, silicon, rubidium, silver, gold, lead, bismuth, titanium, and others. Such elements are believed to be acquired by the animal body as environmental contaminants and to reflect the contact of the organism with its environment. It was suggested that the difference in distribution patterns of the essential and non-essential elements in living organisms might be explained by different ways of incorporation and retention. For the essential elements an internal control mechanism is postulated, leading to a normal or symmetrical distribution. For the nonessential elements, arising from contamination and having no significant function, external control of tissue concentration would occur, leading to a distribution pattern similar to the environmental level.

The evolution of industry based on the use of metals which culminated in the Industrial Revolution has as one of its side effects contamination of the globe with certain metals found naturally in low concentrations. Unlike the elements essential for life or health some of these metals are inherently toxic: cadmium, lead, mercury, antimony, beryllium. Living things have evolved in the presence of very low concentrations of these toxic elements, and the need for detoxifying systems has been relatively unnecessary -- until the present. Now, as best can be ascertained, the body burdens of industrialized urban man are elevated with respect to three elements:

lead, cadmium, and arsenic, certainly the result of modern industrial practices. Therefore, it would not be surprising if some chronic diseases resulted from metabolic breakdown coming from accumulation of an abnormal toxic trace element during a lifetime.

The common chronic diseases of our civilization are atherosclerosis, hypertension, arthritis, cancer, diabetes, and cirrhosis of the liver; these diseases are the leading causes of death and disability. Some of these disorders are at least partly affected by dietary factors. Therefore, the relation of trace elements -- deficiencies of essential ones and excesses of toxic ones -- to common chronic diseases has been investigated extensively. For example, the World Health Organization has been conducting a large survey on cardiovascular disease and trace elements in tissues and in the environment. Enough evidence, both indirect and direct, has now accumulated to involve trace elements as causal factors of two cardiovascular diseases, atherosclerosis and hypertension.

A possible role of cations in hypertension was suggested by a number of investigators. Schroeder and Perry (1955) noted that, except for ganglionic blocking agents, all known antihypertensive agents had "an affinity for certain trace metals". They postulated that the antihypertensive action might be due to this metal-binding capacity. Measurements of the urinary excretion and tissue concentrations of certain trace metals in people with or without hypertension in several geographic areas supported this hypothesis. Among the various elements, notably vanadium, cadmium, lead and manganese

appeared to be the most suspect in human "essential" hypertension. Comparing the mortality rates in the United States from cardiovascular diseases (including hypertension) with hardness of public water Schroeder (1960) concluded that something "associated with the nature of public water supplies affects adversely the course of degenerative cardiovascular disease in the United States".

Schroeder conducted experiments on rats fed for life small doses of each of 20 metals in drinking water, in a metal-free wood animal laboratory (Schroeder et al. 1963). Blood pressure, heart weights, and microscopic sections were evaluated. The only trace metal found to cause hypertension was cadmium (Schroeder, 1964a).

The element cadmium (Cd) was discovered in Gottingen by Stromeyer who noted yellow tinge in a sample of iron-free zinc carbonate which he attributed to a new element (Prodan, 1932). Cadmium is closely related to zinc and is always found with zinc in nature, cadmium is obtained as a by-product in the refining of zinc and other metals. However, as it is difficult to separate zinc and cadmium, the latter will often be found in small amounts in commercially available zinc compounds (Schroeder et al., 1967).

In this century cadmium and cadmium compounds have been used increasingly by industries, causing a sharp increase in environmental pollution. Cadmium is delivered to air, soil and water by mines, by metal smelteries, and by industries using cadmium in alkaline accumulators, alloys, paints, and plastics. The burning of oil, waste and scrap metal treatment also contributes to environmental

contamination with cadmium ... as does the use in agriculture of fertilizers, either as chemicals or as sludge from sewage plants, and the use of cadmium-containing pesticides. Some of the cadmium in the air is inhaled by humans and animals, but most of it is deposited in soil or water. The cadmium deposited in water then increases, the concentrations of cadmium in edible water organisms. In the event of flooding or irrigation, cadmium in the water increases the concentration in soil, in turn causing an increase of cadmium in agricultural products, such as rice and wheat.

Schroeder (1964b) suggested that at least part of human "essential" hypertension was associated with inapparent cadmium exposure. He based this hypothesis on two major premises: first, that man has a low but continuous exposure to environmental cadmium which results in considerable accumulation of the metal in virtually all adult human kidneys (Schroeder and Balassa, 1961), with hypertensive subjects apparently having abnormally high concentrations (Schroeder, 1965). Second, when Long-Evans rats were given traces of cadmium in drinking water (5 mg/L) from the time of weaning, hypertension began to appear after about a year and increased in incidence with age. Once hypertensive the rats remained so until death (Schroeder, 1964a; Schroeder and Vinton, 1962). This disorder was similar to its human counterpart, in that the incidence in females was greater than in males, the mortality among males with hypertension was greater than that among the females, both sexes had decreased median life spans compared to cadmium-free controls (Schroeder et al., 1965), aortic lipid content

was increased and the incidence of spontaneous aortic plaques was sizeable (Schroeder and Balassa, 1965), although less than in the human disease. Hearts were enlarged, renal arteriolar sclerosis was found frequently, considerable cadmium was present in kidneys and the systolic blood pressures frequently exceeded 260 mmHg (Schroeder, 1964a). Using conditions very similar to Schroeder's, Perry and Erlanger (1974) found that 1, 2.5 and 5 mg cadmium per liter of drinking water reproducibly induced hypertension in Long-Evans rats after one year. Furthermore, Perry and Yunice (1965), found that an intra-arterial injection of 0.1 to 0.4 mg cadmium per kilogram caused increased blood pressure in Sprague-Dawley rats, whereas larger doses (0.8 to 3 mg Cd/kg) resulted in a decreased blood pressure. Schroeder et al. (1966) found an increase in the systolic pressure after an intraperitoneal injection of 1 to 2 mg cadmium per kilogram in Long-Evans rats. Hypertension was produced in Wistar rats by intravenous injections of cadmium in doses of 0.02 to 2 mg/kg (Perry et al., 1970) and by intraperitoneal injections of 0.2 to 2.4 mg/kg (Perry and Erlanger, 1971).

There are also negative findings in regard to cadmium and hypertension. Morgan (1969) determined renal cadmium in autopsy cases and did not find differences between a group with hypertension and a control group. Lewis et al. (1972) reported that cadmium concentration in autopsy samples of kidney, liver, and lung were correlated to smoking habits and that this association was not affected by the presence of arterial hypertension. In 1969, Tsuchiya reported a

unique disease called "Itai-Itai Byo" affecting some residents of the Toyama area of Japan (Tsuchiya, 1969). The main features of the fully developed syndrome include pain in bones, waddling gait, aminoaciduria, glycosuria, altered pancreatic functions, and severe osteomalacia. It was concluded that the cause of the disease was related to exposure to cadmium from a nearby factory. However, hypertension has not been found in patients with Itai-Itai Byo disease, nor in people living near the endemic district where the disease is found, nor in people living in other cadmium-contaminated areas of Japan.

Lener and Bibr (1970; 1971) reported that hypertension did not appear when female Wistar rats were given cadmium in drinking water (5 mg/L) and observed for 16 months. Castenfors and Piscator (unpublished data), using female Sprague-Dawley rats, could not induce hypertension by giving them injections of cadmium (0.5 mg/kg) 3 days a week for 6 months. In another experiment the rats were given cadmium in drinking water (5 mg/L) for 1 year, but monthly determinations of blood pressure did not show any difference compared with controls. Doyle et al. (1975) showed that 5 mg cadmium per liter of drinking water did not produce hypertension in male and female Long-Evans rats after 340 days of treatment. Furthermore, Porter (1974), using female Long-Evans and male and female Sprague-Dawley rats, was unable to obtain hypertension following intra-peritoneal cadmium (0.21, 0.42, 0.84 or 2 mg/kg) administration. Therefore, the failure of various epidemiologic studies to confirm a clear relation between cadmium intake and hypertension made

Schroeder's hypothesis doubtful.

The role of salt consumption in human "essential" hypertension has been a controversial subject for some years. Two of the basic observations that led to the concept are concerned on the one hand with the observation that restriction of sodium chloride (NaCl) often ameliorates "essential" hypertension in man and on the other hand that excess consumption of NaCl produces the disease experimentally in some animals. The first suggestion that salt intake and hypertension in man are connected stems from Ambard and Beaujard (1904). Kempner's rice-fruit diet was the first large scale, successful attempt to influence hypertensive disease by dietary treatment (Kempner, 1944). It has been shown that its effect was due primarily to its low sodium content (Dole et al., 1950). Dahl considered the possibility that salt might be a cause of "essential" hypertension and during the years 1954-1960 accumulated evidence in support of this thesis, (Dahl, 1958; Dahl, 1960; Dahl and Love, 1954; 1957, Dahl et al., 1960). There is a considerable body of data which indicates that salt plays a central role in certain forms of experimental hypertension as well. The first experimental evidence that salt might play a role in the development of hypertension was reported by Grollman et al. (1940); they observed that various sterols would produce hypertension in the rat only if additional salt were added. During the next 10 years their observation was confirmed repeatedly. Between 1948 and 1950 a number of observers reported that hypertension could be induced in

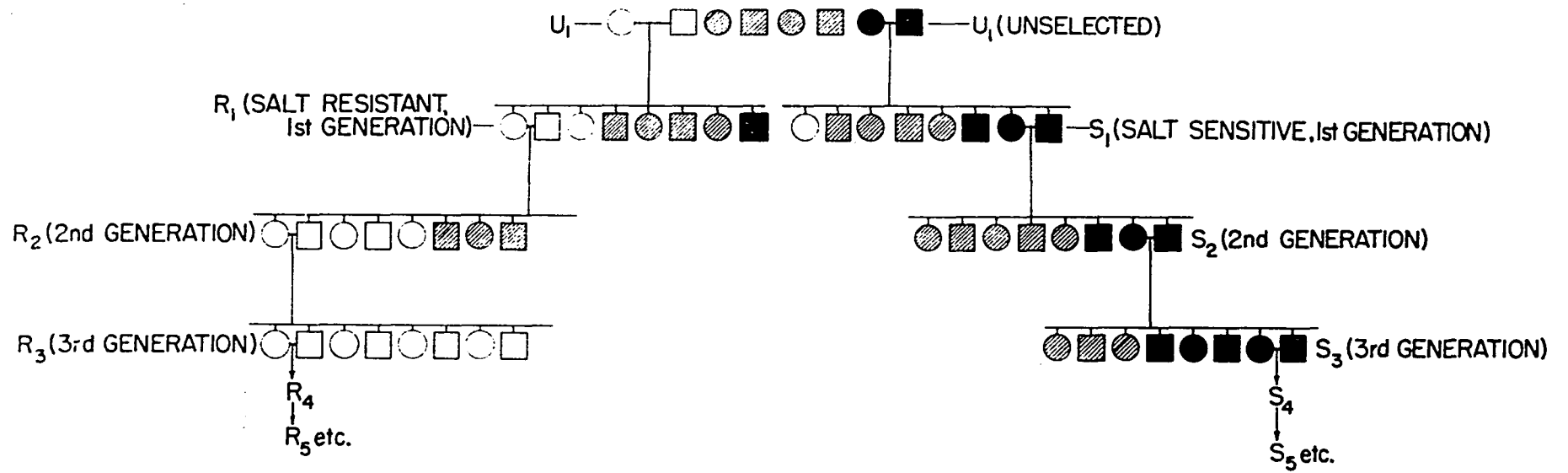
several animal species by substituting hypertonic saline for drinking water. Finally, in 1953 Meneely and his associates (Meneely et al., 1953) reported that excess salt consumption in food caused hypertension in rats.

If chronic salt ingestion played a causal role in hypertension, it was fair to raise the question why did not every person or rat, eating large amounts of salt become hypertensive? During 15 years, Dahl noticed that among 4,000 rats all of which were exposed to the same environment including a high salt diet, some never developed hypertension whereas a few developed fulminating hypertension within a month or two after starting the diet. He suspected that these marked variations in response to high salt diet might be controlled by genetic factors. If this were true, by using the technique of selective inbreeding, it should be possible to evolve two strains of rats whose blood pressures would differ sharply after salt ingestion. At Brookhaven National Laboratory, in 1960, Dahl and his co-workers initiated studies which have now led to the development of two distinct strains of rats - Dahl hypertension-resistant or R-strain and hypertension-sensitive or S-strain - the members of which show strikingly different blood pressure responses to chronic salt ingestion and various other hypertensinogenic stimuli.

For details, the original papers may be consulted (Dahl et al., 1962a; 1962b), but a schematic summary of the technique used by Dahl and associates for selective inbreeding is shown in Figure 1. An unselected (U_1) group of Sprague-Dawley rats, received as weanlings

FIGURE 1

Schematic representation of selective inbreeding from successive generations of rats.



BP(mmHg)	MALE	FEMALE
<140		
140-179		
180 ⁺		

from a supplier, was used initially. Among the animals in this group, those that responded to the regimen with the highest (the Sensitive or S-strain) and lowest (Resistant or R-strain) pressures, respectively, were selected for further breeding, and the remaining animals were discarded. The sensitive animals so selected were mated and their offspring were designated as the S_1 generation; the offspring from the first mating of resistant animals were designated as the R_1 generation. From these S_1 and R_1 animals, and similarly in succeeding generations, only those that responded to the regimen with the highest and lowest pressures, respectively, were selected for breeding the two strains. After three generations of such selective inbreeding the response of the two strains to salt was significantly different. All high salt-fed animals consumed the same diet (8% NaCl) and in similar amounts; yet, among such R_3 animals, no significant elevation of pressures was observed whereas the overwhelming majority of the S_3 rats were hypertensive and many of them severely so. By contrast, the S_3 animals maintained on the control (low salt) diet (0.4% NaCl) did not develop hypertension. This suggested that, while the S_3 strain had a strong genetic predilection to develop hypertension, the addition of a high salt diet was necessary to unmask the trait. Among the R_3 animals, excess dietary salt seemed to have little influence on the blood pressure response. Comparison of the response of males with that of females on the same regimen indicated that, in each instance, the mean blood pressure of the males was significantly higher than that of the females. Most animals in the S strains now rapidly develop hyper-

tension; indeed, we have seen animals in these latest generations that died with fulminating hypertension three weeks after beginning a high salt diet; between one-quarter and one-half are dead within three months. The rate and the degree of blood pressure increase as well as the malignancy of disease afflicting S rats depend on the amount of salt added and the age at which it is started. The heavier the exposure, and the earlier in life it begins, the more malignant the disease.

The same genetic difference between R and S strains of rats was observed when other techniques were used to induce experimental hypertension (Dahl et al., 1963; 1965; Friedman and Iwai, 1976; Iwai et al., 1969): Deoxycorticosterone acetate plus NaCl; unilateral renal artery constriction without NaCl; adrenal regeneration with NaCl; cortisone without NaCl; uninephrectomy without NaCl; and psychological stress. Thus, the genetic background critically modified the response to procedures used to induce at least six "varieties" of experimental hypertension. Accordingly it seemed reasonable to investigate the genetic influence on the effects of cadmium on blood pressure and other parameters in Dahl strains of hypertension-resistant and hypertension-sensitive rats.

II. MATERIALS AND METHODS

A. The Rats and their General Care:

All the rats were selected from one of two unique strains of rats originally derived from a common pool of Sprague-Dawley ancestors by selective inbreeding. They were designated Dahl hypertension-resistant strain, or R, and Dahl hypertension-sensitive strain, or S, respectively, because of their resistance or susceptibility to develop hypertension in response to chronic excess salt ingestion as well as to other experimental procedures.

All animals were housed in stainless steel cages, 1-5 animals per cage depending upon sex ("small" cages, 425 cm² floor space held one male or two females; "large" cages, 991 cm² floor space held four males or five females). The racks were automatically flushed at 1/2-h intervals (Hoeltge, Inc., Cincinnati, Ohio) with a watering system (Hardco Scientific Div., Fieldstone Corp., Cincinnati, Ohio). The cages were in air-conditioned rooms maintained at 22° C (± 1° C) and 50% relative humidity with artificial light on for 9 h and off for 15 h. Stringent precautions were taken to avoid unnecessary metallic contamination. The animals were not manipulated or disturbed except for (1) blood pressure measurement and (2) blood and urine collection for chemical analysis.

B. The Rat Food and Drinking Water:

Since the presence of zinc and calcium may be of major significance of the production of experimental hypertension with cadmium (Schroeder and Buckman, 1967; Schroeder et al., 1967) and

may also account for conflicting results (Perry and Erlanger, 1974), dietary levels of calcium and zinc were carefully monitored.

Rat Food. All studies were carried out with the rats on special low salt (0.4% NaCl) food (Agway, Inc., Country Foods Division, Syracuse, N.Y.) fed ad libitum. To the basic rat food various specified concentrations of NaCl were added at the factory, and all shipments were analyzed for their NaCl. The approximate chemical composition of the rat food reported by Dr. M. C. Stillions of Agway, Inc., is listed below:

Amino Acid Content (%)

Protein	23.20
Arginine	1.57
Lysine	1.51
Methionine	0.43
Cystine	0.38
Tryptophan	0.31
Glycine	1.27
Histidine	0.54
Leucine	1.93
Isoleucine	1.27
Phenylalanine	1.09
Threonine	0.93
Valine	1.26
Fat (%)	4.30
Fiber (%)	5.20

Mineral Composition (Ash %)

Cadmium	0.08 parts per million (ppm)
Calcium	0.75%
Phosphorous	0.86%
Potassium	1.51%
Lead	0.41 ppm
Manganese	35.00 ppm
Magnesium	0.25%
Nickel	0.16 ppm
Iron	172.00 ppm
Iodine	0.31%
Cobalt	0.07%
Copper	13.50 ppm
Zinc	40.00 ppm
Sodium Chloride	0.40%

Vitamin Content (per pound)

Vitamin A	16.79 IU
Vitamin D suppl.	500.00 IU
Vitamin E suppl.	41.00 IU
Vitamin B ₁	2.90 mg
Vitamin B ₆	0.38 mg
Vitamin B ₁₂	4.35 µg
Niacin	7.61 mg
Pantothenic Acid	1.01 mg
Choline	690.00 mg
Carotene	3.00 mg

Riboflavin 6.50 mg

Ingredients:

Soybean meal	Dried whole whey
Ground wheat	Alfalfa meal
Ground oats	Animal fat (stabilized)
Wheat midds	Brewers dried yeast
Corn meal	Dicalcium phosphate
Calcium carbonate	Fish meal

The low salt (0.4% NaCl) food contained adequate sodium for normal growth and development (Dahl et al., 1962a; 1964).

Drinking Water. The only fresh daily water provided for drinking was tap water which was available to the rats ad libitum in plastic bottles with rubber stoppers and stainless steel drinking tubes. The concentrations of the minerals of interest in the tap water were: sodium (0.5 to 0.7 mEq/L); cadmium (less than 0.003 mg/L); calcium (9 to 11 mg/L); and zinc (0.06 to 0.08 mg/L).

C. Direct Blood Pressure Measurement:

The rat was anesthetized with 1.4 g urethane (Sigma Chemical Co., St. Louis, Mo.) per kilogram of body weight subcutaneously in a concentration of 0.2 g/ml. Thirty minutes later the rat received an injection of 15 mg pentolinium tartrate (Ansolysen, Wyeth Laboratories Inc., Philadelphia, Pa.) per kilogram of body weight intraperitoneally in a concentration of 10 mg/ml. After a further 30-min period tracheotomy was performed, and a PE 20 polyethylene catheter (Clay-

Adams, Parsippany, N.J.) filled with 150 mM NaCl was inserted into the right common carotid artery. Blood pressure was continuously recorded with a pressure transducer (P 23 DC, Statham Laboratories, Hato Rey, Puerto Rico) connected to a polygraph (Model 7, Grass Instruments, Quincy, Mass.). The chart speed was 2.5 mm/min. There was no regulation system for body temperature.

D. Indirect Blood Pressure Measurement:

Systolic blood pressure was measured by tail plethysmography according to the method of Friedman and Freed (1949) by the use of a sensitive microphone on the tail. This method has been found to give reproducible results when animals were anesthetized with ether, using a flowing oxygen-ether mixture, and were warmed in a special box to 40°C for 3 to 5 minutes in order to produce maximal vasodilatation.

Subjective factors in estimating blood pressure were minimized by using two precautions routinely: (1) individual rats were identified (metallic numbered clips attached to the back of the neck) only after blood pressures were recorded; (2) groups of rats were rotated so that the same person did not measure the pressure on a rat at successive sittings. At least four systolic readings were recorded with each measurement, and the average was used for that day. Differences within 10% of the previously recorded pressure of rats with stable pressures were considered acceptable within the range of physiological variation, while greater differences were rechecked at 2-day intervals alternately until satisfactory agree-

ment was reached. The number of the latter instances was less than 1 in 20, and they were randomly distributed among the rats. Blood pressure was measured at one to two week intervals depending on the rate at which the blood pressure was advancing.

Hypertension was defined as a level of blood pressure which was more than 3 standard deviations greater than the mean of the controls for the same age.

E. Body Weight Measurement:

Body weight was recorded concomitantly with each measurement of the blood pressure. The earliest objective sign of ill health was weight loss. If this was in excess of 10 g and occurred between successive blood pressure measurements, it was assumed that the animal was ill -- although at no time did the animals appear to be suffering. The previously recorded pressure was used in the final calculations if this illness proved terminal. Occasionally, intermittent respiratory infections were associated with transient weight loss followed by recovery and subsequent normal weight gain; in these instances, the final blood pressures recorded at the end of the observation period were used for statistical analysis.

F. Determination of Blood Urea Nitrogen:

The procedure used for the determination of blood urea nitrogen (BUN) on the AutoAnalyzer II (Technicon Instruments Corp., Terrytown, N.Y.) was a slightly modified version of the procedure described by Marsh et al. (1965). This method determined BUN by the colored product formed when urea, in relatively weak acid solution, reacts

with diacetyl monoxime in the presence of thiosemicarbazide and ferric ion.

Reagents:

Sodium solution containing NaCl and Brij-35: (Technicon).

Alkaline potassium ferricyanide (Technicon).

Working BUN acid containing ferric chloride, phosphoric acid, sulfuric acid (Technicon).

Working BUN color reagent containing diacetyl monoxime and thiosemicarbazide (Technicon).

Urea standards: 10, 30, 50, 70, 100, 150 mg/100 ml of BUN (Technicon).

Plasma (0.2 ml) or urea standards (0.2 ml) were aspirated in the AutoAnalyzer II at the rate of 60 samples per hour and diluted with sodium solution. This mixture was dialyzed against a sodium solution and the resulting dialysate was added to working BUN color reagent and working BUN acid solutions in a 95° glycerol bath. The pink solution resulting from this reaction was air-cooled in a mixing coil before being passed through a 10-mm flow cell colorimeter equipped with 520-m μ interference filters. Results were calculated by comparison of data obtained for the test solutions with those obtained for standard urea solutions. BUN was expressed as mg BUN/100 ml of plasma.

G. Determination of Plasma Cholesterol:

The procedure used for the determination of cholesterol on the AutoAnalyzer II (Technicon Instruments Corp., Terrytown, N.Y.) was

an adaptation of Rush et al. (1970) method. This procedure was based on the Lieberman-Burchard reaction.

Reagents:

Cholesterol color reagent containing acetic anhydride, glacial acetic acid and sulfuric acid (Technicon).

99% Isopropanol (Technicon).

Zeolite mixture containing zeolite, lloyd reagent, cupric sulfate and calcium hydroxide (Technicon).

Triolein standard (Technicon).

Procedure for Sample Extraction.

(1) 3.8 ml of 99% isopropanol was placed in a screw capped tube (Culture tube 16 x 100 mm, Fisher Scientific Co., Fair Lawn, N.J.) 0.2 ml of plasma was pipetted into the isopropanol. (2) The tube was capped and shaken vigorously for 30 seconds on a vortex mixer (Vortex-Genie, Scientific Industries, Inc., Springfield, Mass.). (3) One g of zeolite mixture was added to the isopropanol extract. The tube was capped and shaken 30 seconds on a vortex mixer. (4) After a batch of samples were prepared, they were allowed to stand for 30 minutes. During this period the entire rack was inverted once every ten minutes and the mixture was gently resuspended. (5) The tubes were centrifuged for 20 minutes and the supernatant was carefully decanted into sample cups.

Procedure for Cholesterol Determination on AutoAnalyzer II:

An aliquot of the extracted plasma was aspirated into an air-segmented stream of the cholesterol color reagent (Lieberman-

Burchard reagent) and heated to 60°C in a heating bath where the development of the color (green) takes place. The absorbance was read at 630 nm in a 15 mm tubular flow cell. The triolein standards (100, 200, 300, and 400 mg/100 ml) were treated in the same manner as the unknowns. Results were calculated by comparison of data obtained for the test solution with those obtained for triolein standards. Plasma cholesterol was expressed as mg cholesterol/100 ml of plasma.

H. Determination of pH, Protein, Glucose and Blood in Urine:

Urine was assayed semi-quantitatively with reagent strips (Latstix, Ames Co., Elkhart, Ind.) for pH, protein, glucose, and blood. A reagent strip was a firm, plastic strip to which was affixed four separate reagent areas for testing for pH, protein, glucose and blood in fresh and uncentrifuged urine. Test results provided information regarding acid-base balance, the status of carbohydrate metabolism, and indications of kidney function (Free and Free, 1972).

Reagents: At the Time of Impregnation:

pH - 0.14% w/w methyl red; 2.8% w/w bromthymol blue; 97% w/w non-reactive ingredients.

Protein - 0.2% w/w tetrabromphenol blue; 99.8% w/w buffer.

Glucose - 16.3% w/w glucose oxidase (1.3 I.U.); 0.6% w/w peroxidase (3300 I.U.); 7.0% w/w potassium iodide; 60.7% w/w buffer; 15.4% w/w non-reactive ingredients.

Blood - 7.1% w/w cumene hydroperoxide; 0.6% w/w orthotolidine; 63.5% w/w buffer; 28.8% w/w non-reactive ingredients.

Chemical Principles of Procedures:

pH Test: This test was based on a double indicator principle which gave a broad range of colors covering the entire urinary pH range. The pH test area permitted, quantitative differentiation of pH values to one unit within the range of 5-9 pH range. Colors ranged from orange (pH 5) through yellow (pH 6) and green (pH 7 and pH 8) to blue (pH 9).

Protein Test: This test was based on the protein error of indicators principle which means that a constant buffered pH, any color development was due to the presence of protein. Quantitative results were obtained from this test area. Colors range from green-yellow for "Negative" through yellow-green (30 to 100 mg/100 ml) to green-blue (300 to 1000 mg/100 ml) for "Positive" reactions. The test area was more sensitive to albumin than to globulin and hemoglobin.

Glucose Test: This was a double sequential enzyme reaction. The reaction utilized the enzyme glucose oxidase to catalyze the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. In turn, a second enzyme, peroxidase, catalyzed the reaction of hydrogen peroxide with the chromogen orthotolidine to form blue oxidized orthotolidine. The glucose reagent area provided qualitative results and was specific for glucose; no substance excreted in urine other than glucose was known to give a positive result. Colors ranged from purple (negative) to purple-violet (positive).

Blood Test: The detection of occult blood was based on the peroxidase-like activity of hemoglobin which catalyzed the reaction

of peroxide and the chromogen orthotolidine, to form blue oxidized orthotolidine. This test area provided semi-quantitative results. Colors ranged from gray (negative reaction) to green-blue (positive reaction). Hemoglobin, myoglobin and erythrocytes gave a positive reaction. The reagent area was slightly more sensitive to free hemoglobin and myoglobin than to intact erythrocytes.

Procedure.

1. The urinary bladder of the rat was emptied by gentle manual pressure and the urine was allowed to wet the reagent areas of the strip.

2. The edge of the strip was tapped against the side of a beaker to remove excess urine.

3. Test areas were compared closely with corresponding color charts on the bottle label at the times specified. The strip was held close to color blocks and matched carefully.

I. Determination of Plasma Renin Activity (PRA):

New England Nuclear (NEN) Angiotensin I [^{125}I] Radioimmunoassay (RIA) kit was used for the in vitro estimation of plasma renin activity (PRA) by the quantitation of generated angiotensin I. The NEN RIA Kit was based on an adaptation of the method of Haber et al. (1969).

1. Reagents Supplied with the NEN Angiotensin I [^{125}I] RIA Kit.

Assay Buffer Concentrate, Charcoal Suspension, Angiotensin I [^{125}I] (approximately 1.3 μCi at calibration date). Angiotensin I Antiserum (lyophilized [Rabbit]). Bovine albumin (lyophilized), Angiotensin I Standards (lyophilized) 5.0, 2.5, 1.0, 0.5, 0.25 and

0.1 ng/ml, Dimercaprol, 8-Hydroxyquinoline Solution, Maleate Buffer Concentrate.

2. Sample Collection.

Venous blood (2 ml) was collected from the jugular vein of the rat which was lightly anesthetized with ether; in a 1-ml disposable syringe containing sufficient ethylenediaminetetraacetate to yield a final concentration of 1 mg/ml. Collected samples were maintained in an ice bath for centrifugation before separating the plasma in a refrigerated centrifuge at 4°C at 10,000 r.p.m. for 15 minutes. The clear plasma was then maintained in an ice bath for immediate assay or stored frozen at -20°C.

3. Preparation of Samples for RIA.

(a) Angiotensin I Generation - General Considerations.

Boucher et al. (1964) demonstrated that the pH optimum of the plasma renin-substrate system is 5.5-6.0. For these reasons, generation conditions in this NEN kit were selected to favor the accumulation of angiotensin I at the pH optimum (5.5-6.0) for renin activity.

(b) Angiotensin I Generation - Specific Protocol.

(i) The frozen samples were thawed in an ice bath, or in a refrigerator at 4°C.

(ii) One ml of each sample was transferred to a clear polyethylene tube (12 x 75 mm, Falcon Plastics, Oxnard, Calif.) in an ice bath and to each was added 10 µl of the dimercaprol solution and 10 µl of the 8-hydroxyquinoline solution.

(iii) Two ml of pH 6.0 maleate buffer was added to each tube.

(iv) Each sample was mixed thoroughly on a vortex mixer. About 1 ml of each sample was transferred to a similarly numbered polyethylene tube. The 1 ml aliquot was incubated for 1 hour at 37°C while the remainder of each sample was maintained in the ice bath for the same period of time.

(v) At the end of the incubation period, all samples incubated at 37°C were matched against their 4°C control in the ice bath and the angiotensin I measured by RIA.

RIA Protocol:

All pipettings were done with the reagents and tubes in an ice bath.

1. A series of 18 tubes were numbered for the standard curve and 4 tubes for each sample to be run.

2. 1600 µl of assay buffer were pipetted into tubes 1 and 2 (Total Count Tubes), and 500 µl tubes 3 and 4 (Blank Tubes).

3. 100 µl of 5% Bovine Albumin were pipetted into tubes 3 and 4 and tubes 5 and 6 ("0" standard tubes).

4. 100 µl of each Angiotensin I Standard were pipetted into the appropriate tubes, i.e., 0.1 ng/ml standard into tubes 7 and 8, 0.2 ng/ml standard into 9 and 10, 0.5 ng/ml standard into tubes 11 and 12, 1.0 ng/ml standard into tubes 13 and 14, 2.5 ng/ml standard into tubes 15 and 16 and 5.0 ng/ml standard into tubes 17 and 18.

5. 100 µl of the samples were pipetted as follows: 4°C control into tubes 19 and 20, 37°C generated samples into tubes 21 and 22. It was continued in this fashion for all samples.

6. 100 μ l of Angiotensin I [^{125} I] were added to all tubes.
7. 500 μ l of Angiotensin I Antiserum were added to all tubes except tubes 1 through 4.
8. All tubes were mixed using a vortex mixer for 5 seconds.
9. The tubes were allowed to incubate at 4°C for 24 hours.
10. Prior to the completion of the incubation, a magnetic stirrer was added to the diluted Charcoal Suspension and active stirring was initiated.
11. At the end of the 4°C incubation all samples were returned to an ice bath and 1.0 ml of the Charcoal Suspension was transferred to each tube.
12. Each sample was vortexed as before and was centrifuged at 10,000 r.p.m. for 15-20 minutes at 4°C.
13. The supernatant fraction of each tube was decanted to appropriately numbered polyethylene tubes for gamma counting.
14. The supernatant tubes were counted using a scintillation well counter (Nuclear-Chicago). Each tube was counted for at least 10 minutes.

Procedure for Calculating the Value of the Unknowns.

After the samples were processed through the RIA, the concentration of angiotensin I generation was determined utilizing the standard (dose-response) curve. This curve was prepared by plotting the percent angiotensin I [^{125} I] bound by each angiotensin I standard versus its respective angiotensin I concentration. The concentration of angiotensin I in the unknown was then determined by comparing the percent angiotensin I [^{125} I] bound to the standard curve.

PRA was expressed as ng/ml/hr of generated angiotensin I. PRA in the unknown was calculated by subtracting the 4°C value from the 37°C value ($\text{PRA} = \text{ng/ml/hr}^{37^\circ\text{C}} - \text{ng/ml/hr}^{4^\circ\text{C}}$).

J. Determination of Cadmium Concentrations in Tissues:

Tissue samples were assayed for cadmium (Cd) by using a Perkin-Elmer Model 503 atomic absorption spectrophotometer equipped with an HGA - 2100 Graphite Furnace and a Deuterium Background Corrector (The Perkin-Elmer Corp., Norwalk, Conn.).

Initial experiments indicated that severe contamination problems existed. Thus, it was necessary to wash all glassware first with concentrated nitric acid (J. T. Baker Chemical Co., Phillipsburg, N.J.). The filter paper (42 Whatman, Beckman Instruments, Inc., Mountainside, N.J.) was also acid washed before use. Wet tissue samples of about 0.1 g were weighed on a balance (Mettler PN 163, Mettler Instrument Corp., Princeton, N.J.) and placed in acid washed beaker. Four ml of concentrated nitric acid was used to dissolve the samples. The samples were digested on a hot plate (Model SP-A1025 Thermolyne Corp. Dubuque, Iowa) in covered beakers at a temperature of about 80°C until only fat remained and the solution volume was reduced to about 1 ml. The samples were then cooled, filtered, diluted to 5 ml with deionized water, and aliquots of 25 µl of each sample were analyzed.

Cadmium standards (0.5, 1, 2.5, 5 and 10 mg/L) were prepared by the dilution of 1000 mg Cd/L standard solution (Fisher Scientific Co., Fair Lawn, N.J.). To obtain accurate results, standards were prepared with about the same acid matrix as was in the samples. To

ascertain whether the range of values obtained was due to variation in cadmium concentration in the sample, lyophilized bovine liver (SRM No. 1577, National Bureau of Standards, Washington, D.C.) was analyzed by the same method. This material was an excellent choice for evaluating the accuracy of a suggested method. It appeared to be quite stable and was relatively easy to dissolve and analyze. The certificate value for cadmium in the bovine liver sample was 0.27 $\mu\text{g/g}$.

The flame method was significantly faster than the flameless method, so whenever the cadmium levels were high enough to use the flame, it was desirable to do so. Standard instrument conditions (Analytical Methods for Atomic Absorption Spectrophotometry, Perkin-Elmer) were used for wavelength, slit width, lamp current, and flame conditions. A cadmium Electrodeless Discharge Lamp (Perkin-Elmer, Norwalk, Conn.) was used during the analysis.

The method of standard additions was a convenient way to compensate for matrix effects while using the Graphite Furnace (Slavin et al. 1975). In this procedure, a known amount of standard was added to an aliquot of the sample in the Graphite Furnace. The increase in absorption was proportional to the amount of standard added, and a direct comparison was made between the sample absorption and this increased absorption due to the added standard.

A Model 056 recorder (Perkin-Elmer) was used to obtain tracings of the samples analyzed.

Cadmium concentration in a tissue sample was expressed as μg cadmium/g wet weight of tissue.

K. Preparation of Tissues for Microscopic Examination:

Tissues were fixed in 10% formalin (formaldehyde solution obtained from J. T. Baker Chemical Co., Phillipsburg, N.J., approximately 40% formaldehyde gas in water, called formalin, was treated as a 100% solution in making 10% formalin). The fixed specimens were sent to the Histo-Pathology Laboratory (Medical Department, Brookhaven National Laboratory, Upton, N.Y.) where they were embedded in paraffin and sections, 4-microns thick, were stained with eosin and hematoxylin stains. The specimens were examined for morphological abnormalities under a Zeiss light microscope. The observers were not informed of the experimental groups from which the sections were originated until all slides were examined.

L. Statistical Analysis of the Data:

Tests for significance were made with Student's t-test or chi-square where applicable. Analysis of variance was made with special computer programs. A CDC 6600 digital computer was used for the analysis. The values shown were either the mean or the mean $\pm$ standard error of the mean (SEM). Data with p value of < 0.05 were considered significant. All p values < 0.001 were assigned that nominal value.

III. EXPERIMENTAL DESIGN

A. Acute Cadmium Study:

Dahl et al. (1964) reported earlier that S-strain rats had significantly greater vascular reactivity to vasoconstrictor substances such as angiotensin II (AII) and norepinephrine (NE) than did R-strain rats. Since it has been shown by Perry and Yunice (1965) that intra-arterial injections of cadmium (Cd) produced prompt but transient increases in the blood pressure (BP) of rats, the goal of the present experiment was to determine if cadmium differentially affected these 2 specially-bred strains of rats. Specifically the two points addressed in this study were: (1) acute effects of intra-arterial cadmium (0.1 and 0.4 mg/kg of body weight) on blood pressure; and (2) effects of cadmium (0.4 mg/kg) on blood pressure responses to intra-arterial injected angiotensin II (20 and 40 ng/kg of body weight) and norepinephrine (200 and 400 ng/kg of body weight).

Protocol.

Thirty-six female rats (18 R and 18 S) weighing from 200 to 250 g, maintained on tap water and low salt food containing 0.4% NaCl, were prepared for direct blood pressure measurements, (1) A polyethylene catheter (PE 10, Clay-Adams, Parsippany, N.J.) filled with 4 mg of heparin (500 USP units, The Upjohn Co., Kalamazoo, Michigan) per ml of 150 mM NaCl (Sodium Chloride, J. T. Baker Chemical Co., Phillipsburg, N.J.) was inserted about 2.5 cm through the left femoral artery. The position of the catheter tip was verified by autopsy. Following the intra-arterial injection of 0.1 ml of the heparin solution (2 mg/kg)

the catheter was filled with 150 mM NaCl solution. After the blood pressure became stabilized for 20 minutes, and initial blood pressure was recorded, the standard procedure was to give a control injection of 0.1 ml of 150 mM NaCl prior to cadmium administration. Cadmium acetate (Fisher Scientific Co., Fair Lawn, N.J.) was dissolved in 150 mM NaCl (0.41 and 1.63 mg Cd/ml of 150 mM NaCl) and injected via the intra-arterial (left femoral) catheter. The rats received either 0.1 mg Cd/kg (6 R and 6 S) or 0.4 mg Cd/kg (12 R and 12 S). Cadmium was always given in a volume of 0.1 ml followed by 0.1 ml of a 150 mM NaCl solution. The total time of injection and subsequent flushing was 15-20 seconds during which blood pressure was recorded.

(2) Angiotensin II (Hypertensin, Ciba Pharmaceutical Co., Summit, N.J.), norepinephrine (Arterenol, Calbiochem. San Diego, Calif.) and cadmium chloride (Fisher Scientific Co., Fair Lawn, N.J.) were dissolved in 150 mM NaCl solution and kept in polyethylene tubes (17 x 100 mm, Falcon Plastics, Oxnard, Calif.). The norepinephrine solution contained in addition 1 mg/ml L-ascorbic acid (Fisher Scientific Co., Fair Lawn, N.J.). The angiotensin II solution comprised 50 and 100 ng AII/ml of 150 mM NaCl (20 and 40 ng AII/kg). The concentrations of norepinephrine solutions were 500 and 1000 ng norepinephrine (as the bitartrate hydrate) per ml of 150 mM NaCl (200 and 400 ng NE/kg). The cadmium solution contained 1.63 mg Cd (as the chloride) per ml of 150 mM NaCl (0.4 mg Cd/kg). After the blood pressure had become stabilized for 15 minutes, the standard procedure was to inject the rats (12 R and 12 S) with 2 doses of angiotensin II (20 and 40 ng/kg) and norepinephrine (200 and 400 ng/kg) via the left femoral artery at

5 to 10 minute intervals. This sequence was repeated three times, followed by 0.4 mg Cd/kg with repetition of angiotensin II and norepinephrine injections. The test materials were always given in a volume of 0.1 ml followed by 0.1 ml of 150 mM NaCl solution. Control injections of 150 mM NaCl solution were administered prior to and after angiotensin II, norepinephrine and cadmium injections. The total time of injection and subsequent flushing of test materials was 15-20 seconds during which blood pressure was recorded.

B. Chronic Cadmium Study 1:

Schroeder et al (1966) reported the induction of hypertension in 120-day-old, female, hooded Long-Evans rats which received 2 doses of cadmium (1 and 2 mg/kg of body weight). This study was performed to determine if the injection of cadmium (Cd) would induce hypertension in weanling male and female R and S rats on low salt (0.4% NaCl) diet.

Protocol.

This study involved sixty weanling (3 weeks of age) rats divided into 2 groups as follows: (a) cadmium (5 male R, 10 female R, 5 male S, 10 female S); and (b) controls (5 male R, 10 female R, 5 male S, 10 female S). The rats in the experimental group were injected with 2 mg of cadmium per kilogram of body weight, as the acetate (Cadmium Acetate, Fisher Scientific Co., Fair Lawn, N.J.) intraperitoneally in a concentration of 1 mg Cd/ml of 0.9% aqueous NaCl on week 3 (3 weeks of age) and followed by second and third injections of 1 mg Cd/kg on weeks 6 and 28. R rats received an additional injection of 1 mg Cd/kg on week 25. The controls received the same volume of 0.9% aqueous NaCl (saline) intraperitoneally. Low salt (0.4% NaCl) food and tap

water were available ad libitum. Systolic blood pressure of experimental and control rats was measured indirectly under light ether anesthesia by tail plethysmography at week 6, and then at one to two week intervals depending upon the development and severity of hypertension. Body weights were recorded concomitantly with blood pressures. At necropsy, both kidneys and liver were removed trimmed, blotted and weighed on an analytical balance (Mettler PN 163, Mettler Instrument Corp., Princeton, N. J.).

Kidneys were further bisected across the short axis. Two halves, one from each kidney, were fixed in formalin for light microscopy and the other two kidney halves and the liver were placed in plastic vials (Nalge Corp., Rochester, N.Y.), and frozen at -20°C until needed to be assayed for cadmium by atomic absorption spectrophotometry (Perkin-Elmer 503).

C. Chronic Cadmium Study 2:

This study was undertaken to determine the distribution of cadmium (Cd) in the renal cortex and medulla of female R and S rats at the onset of cadmium-induced hypertension.

Protocol.

Twenty-four weanling (3 weeks of age) rats were divided into 2 groups as follows: (a) cadmium (6 R and 6 S); and (b) controls (6 R and 6 S). The rats in the experimental group were given 2 mg of cadmium per kilogram of body weight, as the acetate intraperitoneally in a concentration of 1 mg Cd/ml of 0.9% aqueous NaCl on week 3 (3 weeks of age) and followed by a second injection of 1 mg Cd/kg on week 6. The controls received the same volume of 0.9% aqueous NaCl (saline)

intraperitoneally. Low salt (0.4% NaCl) food and tap water were available ad libitum. Systolic blood pressure of experimental and control rats was measured indirectly under light ether anesthesia by tail plethysmography on weeks 6 and 8. Body weights were recorded concomitantly with blood pressures. At necropsy, both kidneys and liver were removed, trimmed, blotted and weighed on an analytical balance. Renal cortex and medulla were separated according to the technique of Manthorpe (1973) as follows: The capsule of the kidney was stripped and the collapsed capsule and renal pedicle were cut flush at the renal hilum. After the capsule was removed, the inner and outer sections of the medulla (white and pink portions) were separated from the cortex (brown portion) with fresh single edge blades (Perma Sharp Mfg. Corp., N.Y.). The renal cortex and medulla of both kidneys and a small piece of the liver were weighed and placed in plastic vials and frozen at -20°C until needed to be assayed for cadmium by atomic absorption spectrophotometry (Perkin-Elmer 503).

D. Chronic Cadmium Study 3:

This experiment was designed to investigate whether the development of cadmium-induced hypertension was age-dependent in male and female R and S rats.

Protocol.

Forty R and S rats aged about 16 weeks were divided into 2 groups as follows: (a) cadmium (5 male R, 5 female R, 5 male S, 5 female S); and (b) controls (5 male R, 5 female R, 5 male S, 5 female S). The

rats in the experimental group were injected with 2 mg of cadmium (Cd) per kilogram of body weight, as the acetate intraperitoneally in a concentration of 1 mg Cd/ml of 0.9% aqueous NaCl on week 16 (16 weeks of age) and followed by 2nd and 3rd injections of 1 mg Cd/kg on weeks 19 and 39. R rats received an additional injection of 1 mg Cd/kg on week 35. The controls received the same volume of 0.9% aqueous NaCl (saline) intraperitoneally. Low salt (0.4% NaCl) food and tap water were available ad libitum. Systolic blood pressure of experimental and control rats was taken under light ether anesthesia by tail plethysmography at week 16, and then at weekly intervals. Body weights were recorded concomitantly with blood pressures. At necropsy, both kidneys and liver were removed, trimmed, blotted and weighed on an analytical balance. Kidneys were further bisected across the short axis. Two halves, one from each kidney, and a small piece of the liver were fixed in formalin for light microscopy and the other two kidney halves and the liver were placed in plastic vials and frozen at -20°C until needed for cadmium determination by atomic absorption spectrophotometry (Perkin-Elmer 503).

E. Chronic Cadmium Study 4:

This study was undertaken to determine whether cadmium (Cd) accelerated the morbidity and mortality of salt-induced hypertension in female S rats on high salt (4% NaCl) diet.

Protocol.

This study involved twenty weanling (3 week old) female S rats divided into 2 groups as follows: (a) cadmium (10 S); and (b) controls

(10 S). The rats in the experimental group were injected with 2 mg of cadmium per kilogram of body weight, as the acetate intraperitoneally in a concentration of 1 mg Cd/ml of 0.9% of aqueous NaCl on week 3 (3 weeks of age) and followed by 2nd and 3rd injections of 1 mg Cd/kg on weeks 6 and 23. The controls received the same volume of 0.9% aqueous NaCl (saline) intraperitoneally. High salt (4% NaCl) food and tap water were available ad libitum. Systolic blood pressure of experimental and control rats was measured indirectly under light ether anesthesia by tail plethysmography at week 6, and then at weekly intervals. Body weights were recorded concomitantly with blood pressures. At necropsy, both kidneys and liver were removed, trimmed, blotted and weighed on an analytical balance. Renal and hepatic cadmium concentrations were measured by atomic absorption spectrophotometry (Perkin-Elmer 503) after the tissues were wet-ashed.

F. Chronic Cadmium Study 5:

This study was designed to explore the effect of cadmium (Cd) injection on two-kidney Goldblatt hypertension on male R and S rats on low salt (0.4% NaCl) diet.

Protocol.

Blood pressure of twenty-four male R and S rats, aged about 7 weeks old, were measured by tail plethysmography and they were divided into 2 groups as follows: (a) cadmium (6 R and 6 S); and (b) controls (6 R and 6 S). Constriction of the right renal artery (two-kidney Goldblatt procedure) of rats in both groups was performed by applying a silver clip as follows: The rat was anesthetized with ether and

a 2-cm-long incision was made in the right side. The right renal artery was then stretched by means of a retractor placed between kidney and muscle layer, separated from the renal vein with a pair of tweezers, and the artery was cleaned of connective tissue. A clip, made of sterling silver ribbon (Materials Limited, Englewood Cliffs, N.J.), with an internal diameter of 0.2 mm was applied on the exposed right renal artery as close as possible to the aorta. Care was taken that the artery rested at the base of the slit and that a visible blood flow remained in the artery behind the clip. The left kidney was left intact. After recovery from the operation, the rats in the cadmium group were injected with 2 mg of cadmium per kilogram of body weight, as the acetate intraperitoneally in a concentration of 1 mg Cd/ml of 0.9% aqueous NaCl, whereas the controls received the same volume of 0.9% aqueous NaCl (saline) intraperitoneally. Low salt (0.4% NaCl) food and tap water were offered ad libitum. Systolic blood pressure of cadmium and control rats was repeated at weekly intervals. Body weights were measured concomitantly with blood pressures. At necropsy, both kidneys and liver were removed, trimmed, blotted and weighed on an analytical balance. Kidneys were further bisected across the short axis. Two halves, one from each kidney, and a small piece of the liver were fixed in formalin for light microscopy and the other two kidney halves and the liver were placed in plastic vials and stored at -20°C until needed to be assayed for cadmium by atomic absorption spectrophotometry (Perkin-Elmer 503).

G. Chronic Cadmium Study 6:

The purposes of this study were first: to confirm Schroeder's controversial finding that female Long-Evans rats, which were fed cadmium (Cd) in the drinking water (5 mg/L) for a year beginning at weaning, developed hypertension (Schroeder, 1964a). Second: to indicate the relationship between the dose and the time course of cadmium-induced hypertension.

General Plan:

Groups of female R and S rats received 0, 1, 2.5, 5 and 10 mg Cd/L drinking water and they were fed either low (0.4% NaCl) or high (4% NaCl) salt diet from the time they were weaned. The parameters which were followed included blood pressure (indirect method), body weight, plasma cholesterol and blood urea nitrogen (AutoAnalyzer II) plasma renin activity (radioimmunoassay for angiotensin I), urinary pH, protein, glucose and blood (semi-quantitatively by reagent strips), susceptibility to infection, tumor formation, mortality rate and pathological changes in the kidney and liver (light microscopy) as a function of cadmium exposure, water consumption as an index of cadmium intake, cadmium concentrations in the kidney and liver (atomic absorption spectrophotometry).

Protocol.

Two hundred weanling female R and S rats (100 rats of either strain) were divided into ten groups of 20 (10 R and 10 S per group) as follows:

1. Low salt (0.4% NaCl) diet and 0 mg Cd/L drinking water.

2. Low salt diet and 1 mg Cd/L drinking water.
3. Low salt diet and 2.5 mg Cd/L drinking water.
4. Low salt diet and 5 mg Cd/L drinking water.
5. Low salt diet and 10 mg Cd/L drinking water.
6. High salt (4% NaCl) diet and 0 mg Cd/L drinking water.
7. High salt diet and 1 mg Cd/L drinking water.
8. High salt diet and 2.5 mg Cd/L drinking water.
9. High salt diet and 5 mg Cd/L drinking water.
10. High salt diet and 10 mg Cd/L drinking water.

At 4, 12, 20 and 28 weeks post-weaning, (1) systolic BP of each surviving rat was measured under light ether anesthesia by tail plethysmography; (2) body weights were recorded concomitantly with blood pressures; and (3) the volume of water consumed by each group of rats was noted for 24 hours. Because of some uncertainty regarding wastage, the amount actually drunk was an approximation. In addition, each group of rats was weighed as a group before and after the water measurement in order to calculate the amount of cadmium intake ($\mu\text{g Cd/kg/day}$).

On weeks 4 and 28, 2 ml of blood was obtained by nicking the tail at the end of the BP measurement when the rat was still lightly anesthetized. The blood was placed in a tube containing 100 USP units of dried heparin and was centrifuged at 10,000 r.p.m. for 10 minutes and the plasma was transferred to another sample tube. Plasma cholesterol and BUN were determined on the AutoAnalyzer II. The urinary bladder was emptied by gentle manual pressure and the

fresh and uncentrifuged urine was assayed semi-quantitatively with reagent strips for pH, protein, glucose and blood. In addition on week 28, 2 ml of blood was collected from the jugular vein by the procedure of Boucher et al. (1967) for the determination of plasma renin activity by radioimmunoassay for angiotensin I. On week 29, the majority of rats manifested signs of respiratory infection which affected their blood pressure and body weight. Therefore, the animals were sacrificed and autopsied. The kidneys, liver, heart and adrenals were removed, trimmed, blotted and weighed on an analytical balance. Kidneys were further bisected across the short axis. Two halves, one from each kidney, half of the median lobe of the liver, and any grossly abnormal tissues were preserved in 10% formalin for microscopic examination. The other two kidney halves and half of the median lobe of the liver were frozen for cadmium analysis by atomic absorption spectrophotometry (Perkin-Elmer 503). The date of death was noted for all animals that died during the experiment, and, except for those that were cannibalized, each animal was weighed, autopsied and studied in the same manner as the animals that were sacrificed.

IV. RESULTS

A. ACUTE CADMIUM STUDY:

1. Effect of Intra-Arterial Cadmium Injection on Blood Pressure.

Table 1 demonstrates that neither the initial blood pressure nor the acute rise of blood pressure in response to intra-arterial control injections of 150 mM NaCl (0.1 ml) were statistically different ($p > 0.05$) between R and S rats used in this study.

Mean changes in blood pressure in response to intra-arterial cadmium (0.1 and 0.4 mg/kg) injections are shown in Figure 2. Cadmium (Cd) produced an immediate decrease in blood pressure to below the "zero" baseline (depressor response) followed by a rapid increase to a level above the "zero" baseline (pressor response). There were no differences in depressor and pressor responses to cadmium (0.1 mg/kg) between R (n=6) and S (n=6) rats. On the other hand, the pressor response to 0.4 mg Cd/kg in S rats (n=12) was significantly higher ($p < 0.001$) than in R rats (n=12).

2. Acute Blood Pressure Responses to Pre and Post-Cadmium Injections of Angiotensin II.

Variations in mean rise of the blood pressure to intra-arterially (IA) injected angiotensin II (20 and 40 ng/kg) before and after injection of 0.4 mg Cd/kg are shown in Figure 3. According to absolute values, there was no significant difference ($p > 0.05$) in mean rise of blood pressure between pre and post-cadmium responses to 20 and 40 ng angiotensin II in S rats (n=12). However, post-cadmium blood pressure responses to angiotensin II were significantly depressed ($p < 0.05$) in

TABLE 1. INITIAL BLOOD PRESSURE AND RISE OF BLOOD PRESSURE IN RESPONSE
TO INTRAARTERIAL CONTROL INJECTION OF 150 mM NaCl IN FEMALE
R AND S RATS

STRAIN (N)	INITIAL BP (mm Hg)	BP* RISE (mm Hg)
R (18)	79.4 $\pm$ 3.3 $\square$	3.8 $\pm$ 0.3
S (18)	81.3 $\pm$ 3.1	4.0 $\pm$ 0.4

N = Number of rats.

BP = blood pressure.

* = Response to 0.1 ml of 150 mM NaCl (control injection).

$\square$ = Mean $\pm$ SEM.

FIGURE 2

Mean changes in blood pressure in response to intra-arterial cadmium injections in R and S rats. Twelve (6 R and 6 S) and 24 (12 R and 12 S) animals received 0.1 and 0.4 mg cadmium, respectively. Each bar represents the mean $\pm$ SEM. Cadmium produced an immediate decrease in blood pressure to below the baseline followed by a rapid increase to a level above the baseline.

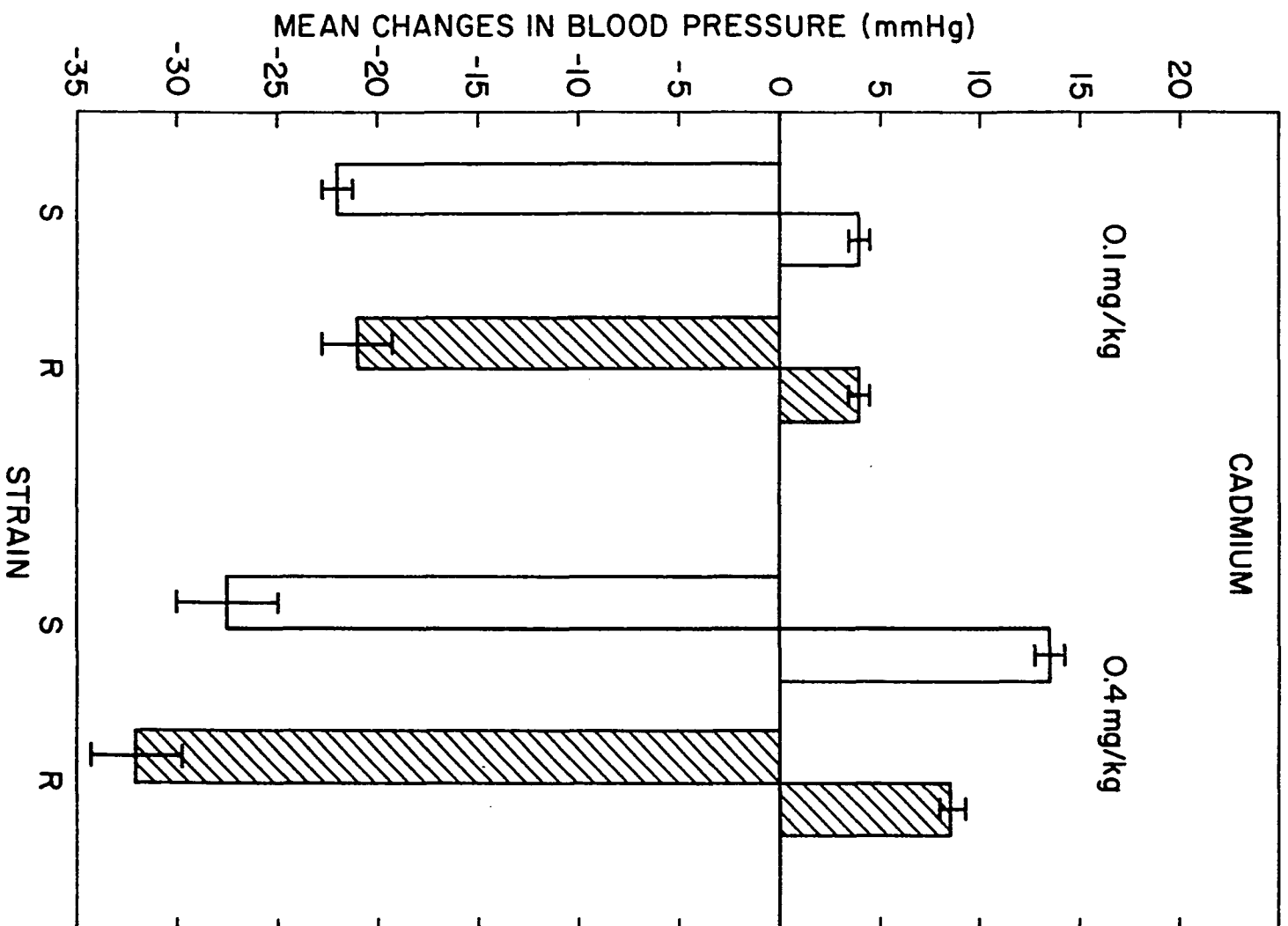
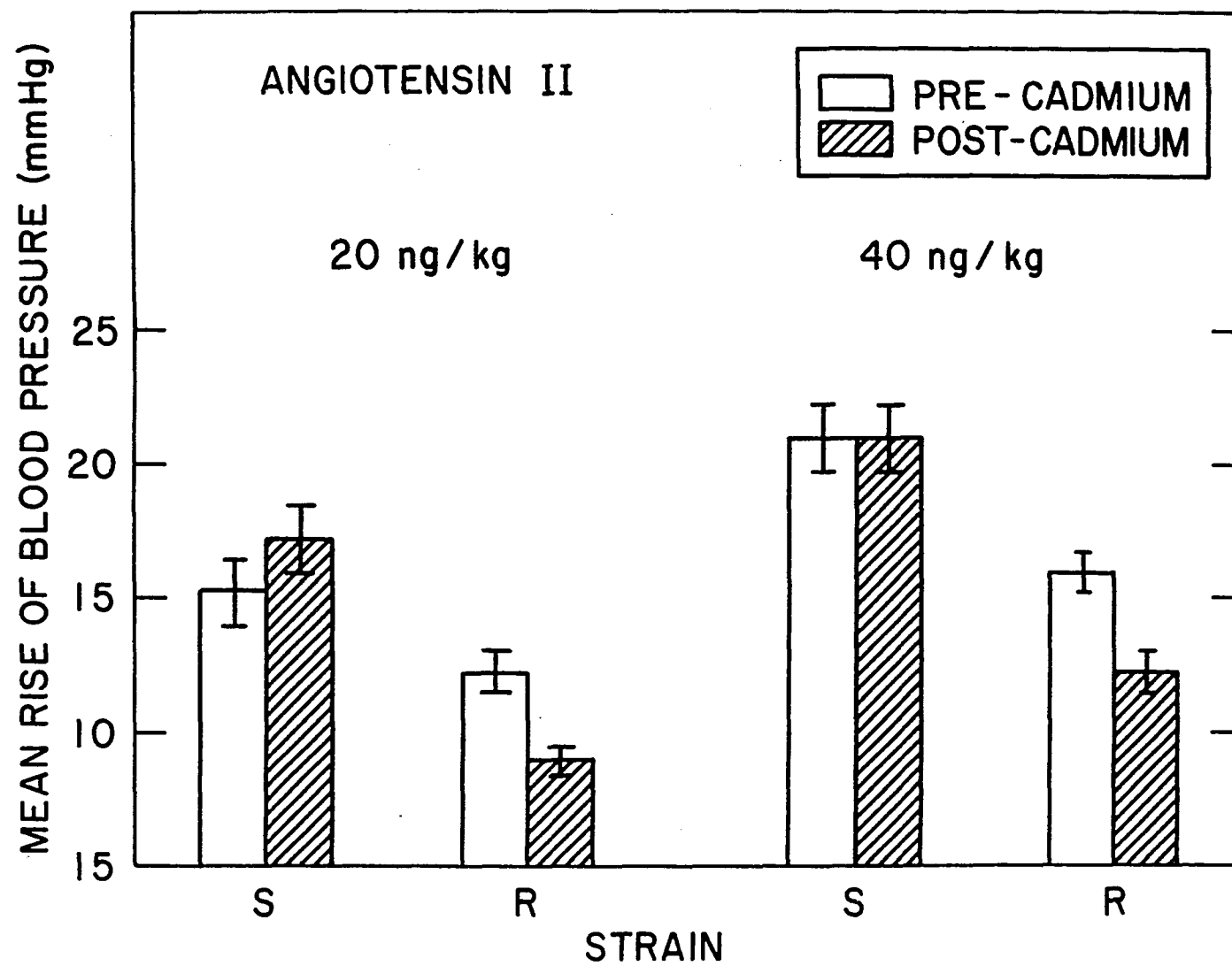


FIGURE 3

Mean rise of blood pressure in response to pre and post-cadmium injections of angiotensin II. Angiotensin II and cadmium (0.4 mg/kg) injections were administered intra-arterially. There were 12 animals of either strain and each bar represents the mean $\pm$ SEM.



R rats (n=12).

3. Acute Blood Pressure Responses to Pre and Post-Cadmium Injections of Norepinephrine.

Figure 4 demonstrates variations in mean rise of the blood pressure to intra-arterially (IA) injected norepinephrine (200 and 400 ng/kg) before and after injection of cadmium (0.4 mg/kg, IA). According to absolute values, there was no significant difference ($p > 0.05$) in mean rise of blood pressure between pre and post-cadmium responses to norepinephrine in S rats (n=12), whereas post-cadmium blood pressure responses to norepinephrine were significantly depressed ($p < 0.05$) in the counterpart R rats (n=12).

Pressor responses to intra-arterial control injection of 0.1 ml of 150 mM NaCl were not affected by cadmium in either strain. Pre and post-cadmium responses to 150 mM NaCl were 3.6 ± 0.3 and 4.1 ± 0.3 (R-strain); 3.5 ± 0.2 and 3.4 ± 0.3 (S-strain) mmHg, respectively.

B. CHRONIC CADMIUM STUDY 1:

1. Effect of Cadmium Injection on Blood Pressure.

The pertinent blood pressure data are summarized in Figures 5 and 6. An analysis of variance indicated the anticipated strain difference with S controls exhibiting significantly higher blood pressure than R controls ($p < 0.001$). But the main objective of this study was to observe the differences in blood pressure between cadmium-injected rats and saline-injected controls within each strain. Among animals exposed to cadmium (Cd), blood pressure, as compared with appropriate controls, was not significantly affected 3 weeks after the first intraperitoneal

FIGURE 4

Mean rise of blood pressure in response to pre and post-cadmium injections of norepinephrine. Norepinephrine and cadmium (0.4 mg/kg) were administered intra-arterially. There were 12 animals of either strain and each bar represents the mean $\pm$ SEM.

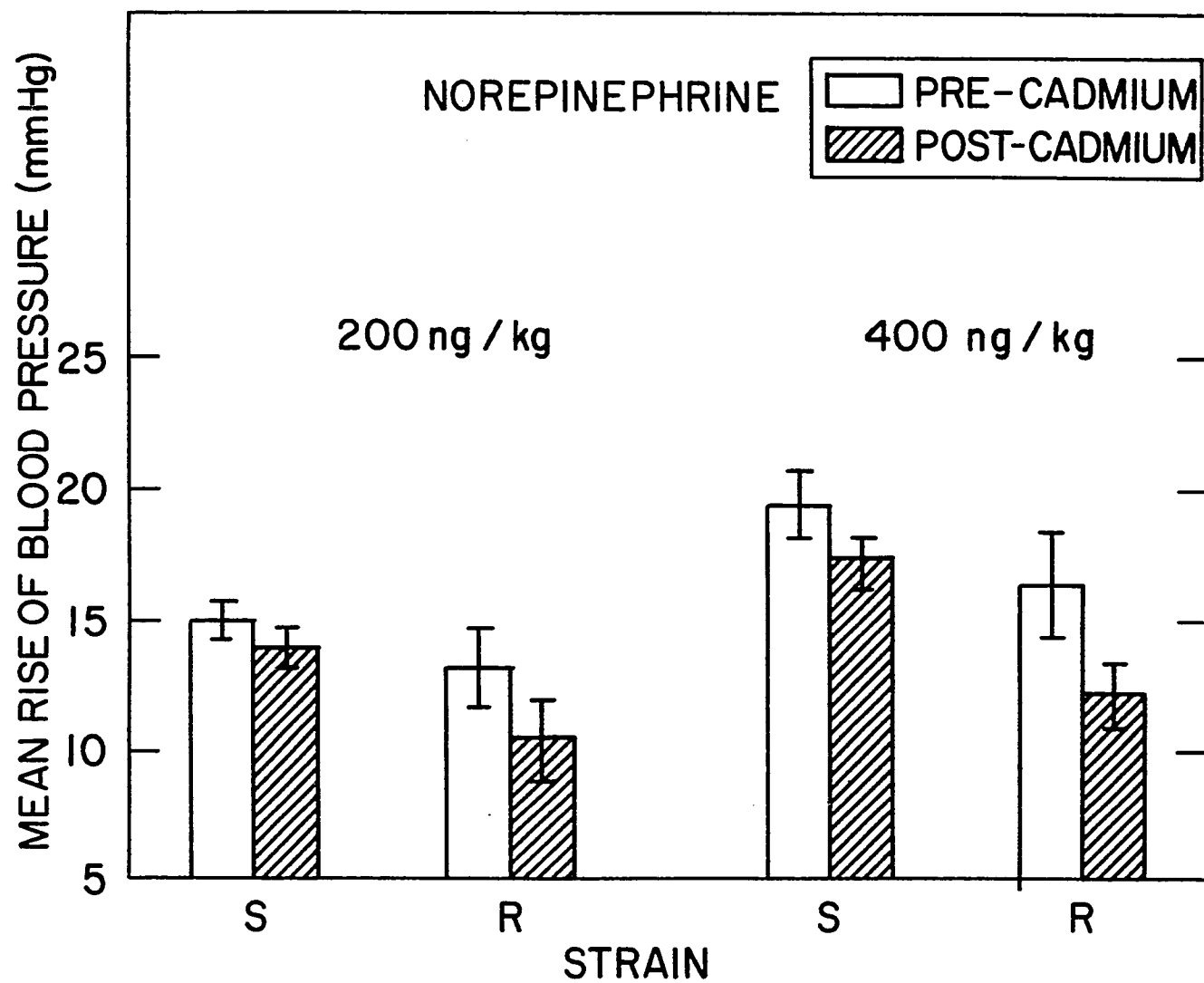


FIGURE 5

Effect of cadmium injection on blood pressure in male R and S rats. Each symbol represents the mean of 5 animals. R and S cadmium rats were given cadmium (2 mg/kg) on week 3 (not shown) and followed by 2nd and 3rd injections of cadmium (1 mg/kg) on weeks 6 and 28. R rats received an additional dose of cadmium (1 mg/kg) on week 25. R and S controls were given saline injections. All injections were given intraperitoneally.

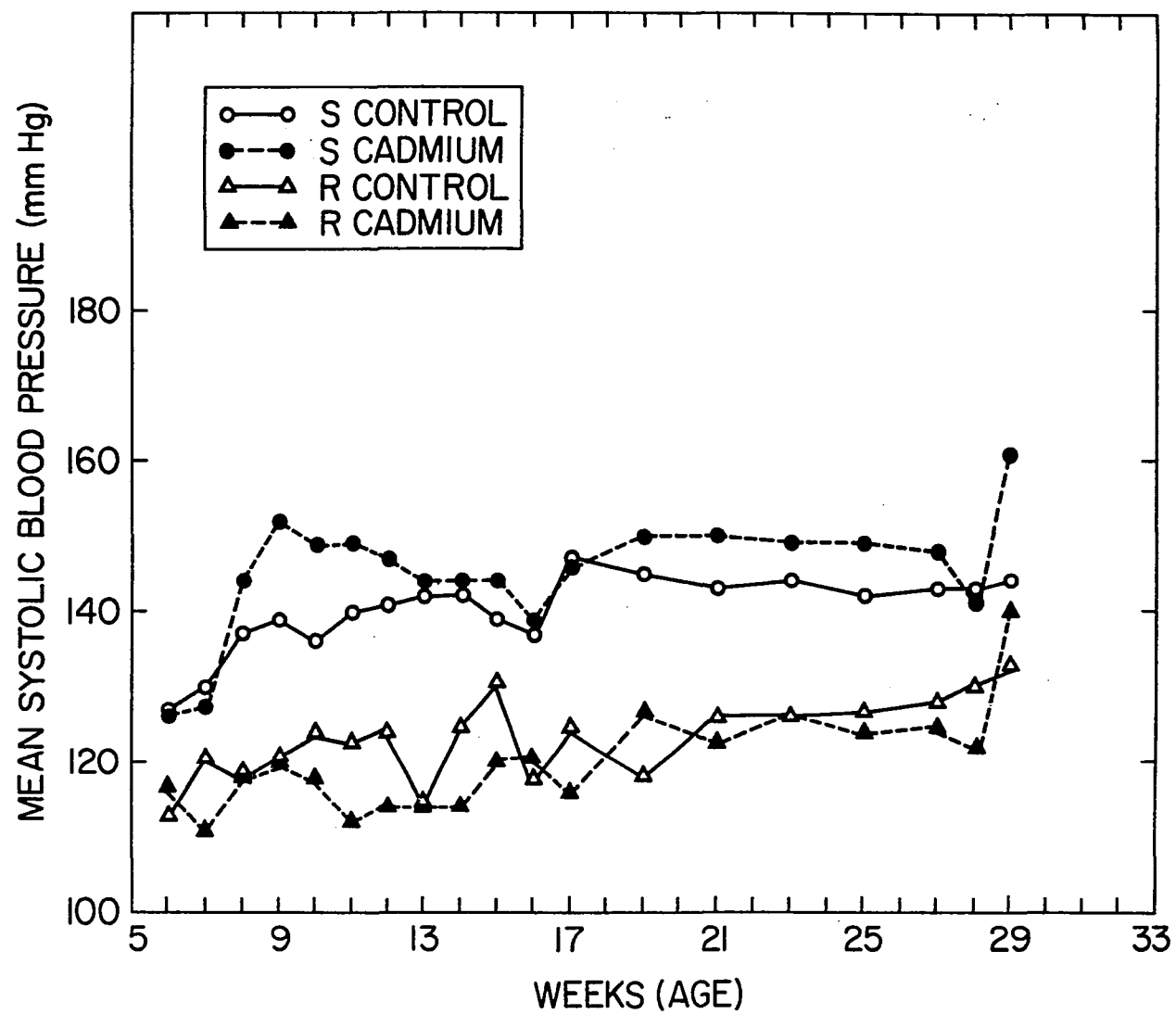
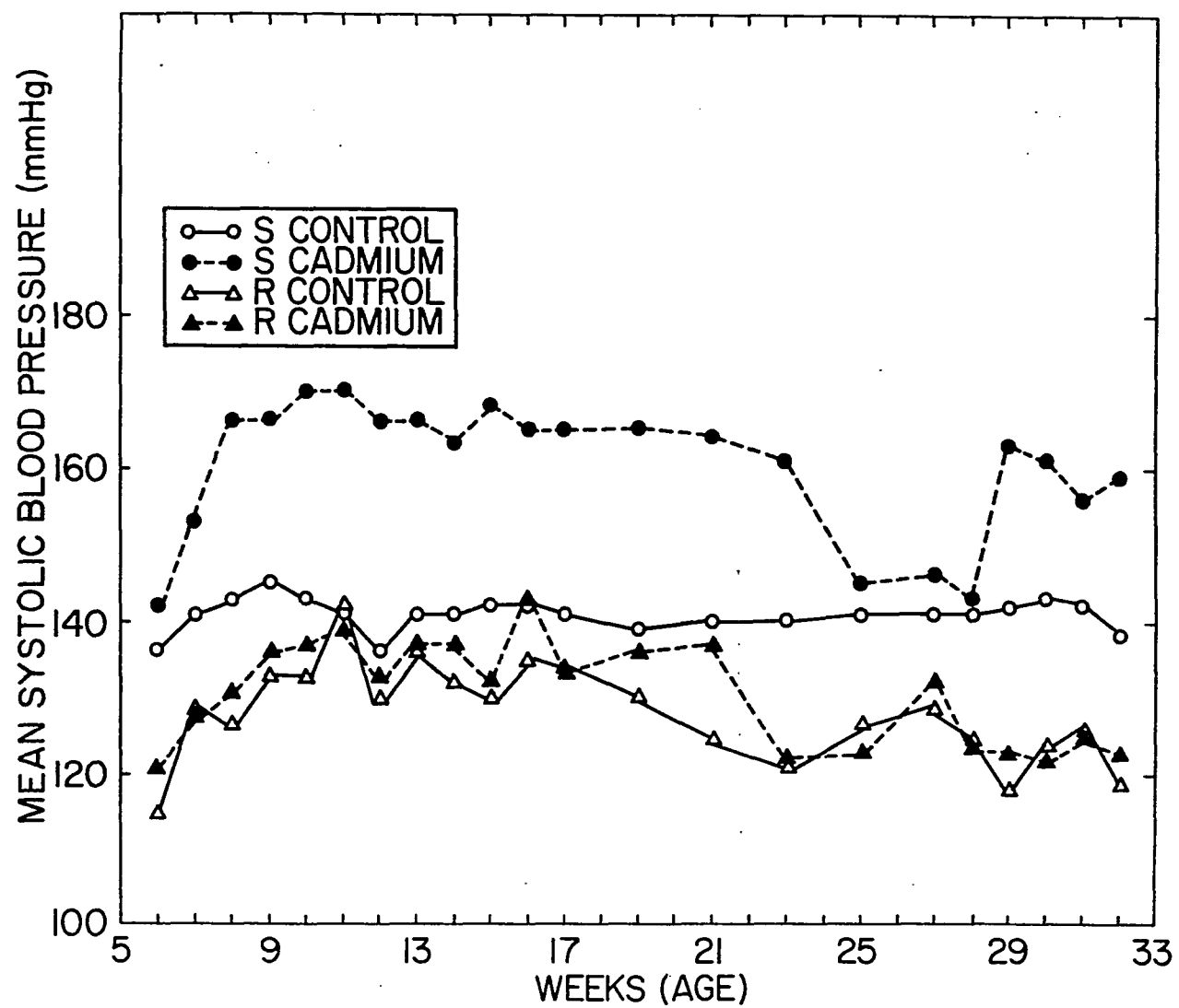


FIGURE 6

Effect of cadmium injection on blood pressure in female R and S rats. Each symbol represents the mean of 10 animals. R and S cadmium rats were given cadmium (2 mg/kg) on week 3 (not shown) and followed by 2nd and 3rd injections of cadmium (1 mg/kg) on weeks 6 and 28. R rats received an additional dose of cadmium (1 mg/kg) on week 25. R and S controls received saline injections. All injections were given intraperitoneally.



(IP) cadmium (2 mg/kg) injection (week 6). Following a second dose of cadmium (1 mg/kg, IP) on week 6, female S rats developed significantly higher ($p < 0.01$) blood pressure (166.8 ± 2.2 mmHg) than their respective controls (143.8 ± 1.1 mmHg) by week 8. The cadmium-induced hypertension continued through week 23, after which the blood pressure returned to control levels (143.7 ± 2.6 mmHg). In response to a third injection of 1 mg Cd/kg given to rats intraperitoneally on week 28 the differences in blood pressure between the female S cadmium and control groups (163.1 ± 1.5 and 142.0 ± 1.9 mmHg, respectively) once again achieved statistical significance ($p < 0.01$) by week 29 and continued thereafter. Male rats, however showed no consistent cadmium-induced blood pressure elevations. Despite an additional injection of cadmium (1 mg/kg, IP) on week 23, R rats remained normotensive throughout the study. None of the R and S controls developed hypertension. Morbidity and mortality commenced among cadmium-injected males and females of both strains on weeks 29 and 32, respectively, and there were no healthy survivors to make a significant comparison thereafter. Of those rats examined post mortem, 90% of the rats in the cadmium group died of massive internal hemorrhage.

2. Effect of Cadmium Injection on Body Weight.

The data are illustrated in Figures 7 and 8. An analysis of body weight showed the expected significant difference between males and females of both strains ($p < 0.001$). However, weights of R and S rats in the cadmium group and their respective controls were not statistically significant ($p > 0.05$). Furthermore, in terms of percent

FIGURE 7

Effect of cadmium injection on body weight in male R and S rats. Each symbol represents the mean of 5 animals. R and S cadmium rats were given cadmium (2 mg/kg) on week 3 (not shown) and followed by second and third injections of cadmium (1 mg/kg) on week 6 and 28. R rats received an additional dose of cadmium (1 mg/kg) on week 25. R and S controls received saline injections. All injections were administered intraperitoneally.

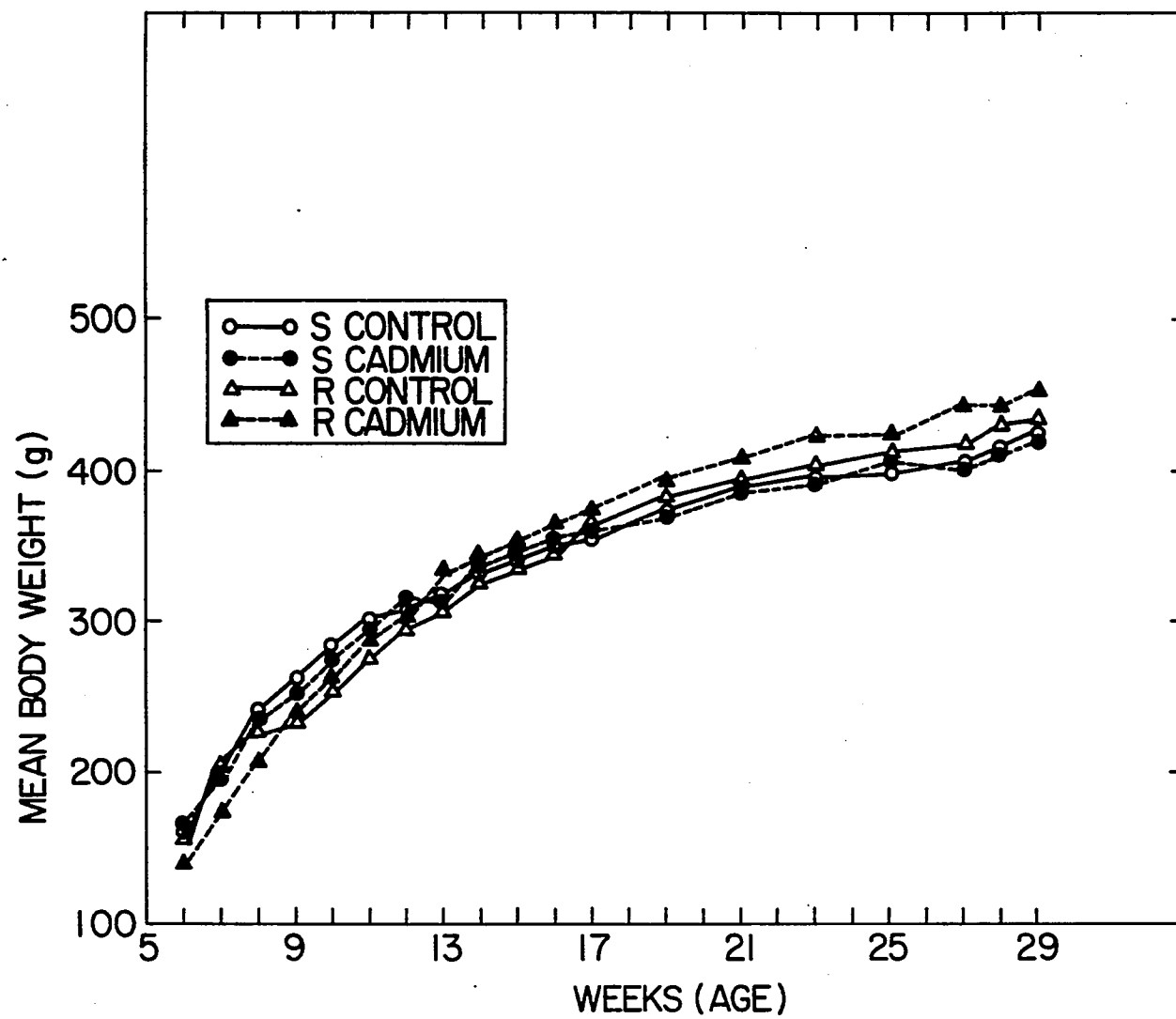
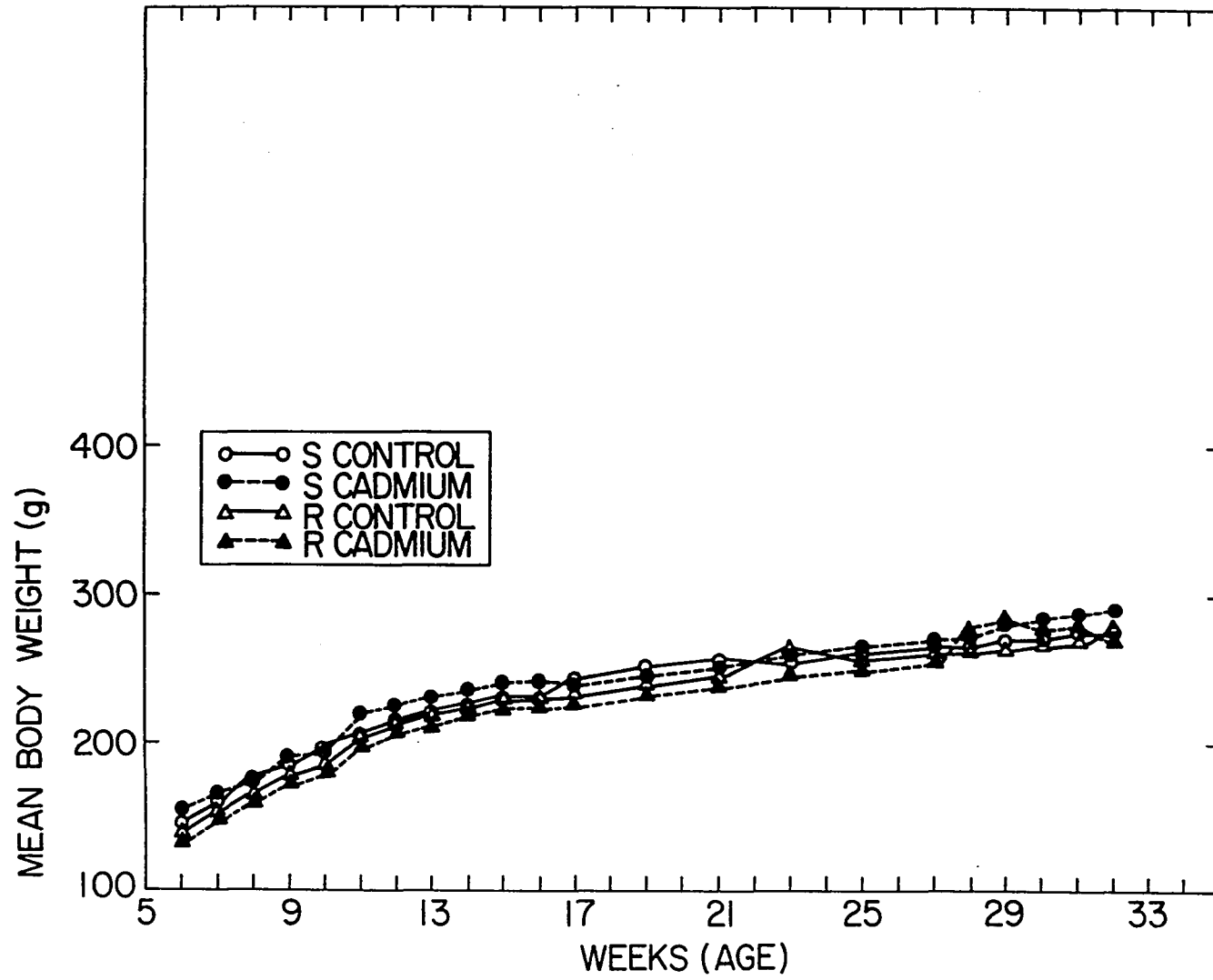


FIGURE 8

Effect of cadmium injection on body weight in female R and S rats. Each symbol represents the mean of 10 animals. R and S cadmium rats were given cadmium (2 mg/kg) on week 3 (not shown) and followed by second and third injections of cadmium (1 mg/kg) on week 6 and 28. R rats received an additional dose of cadmium (1 mg/kg) on week 25. R and S controls received saline injections. All injections were given intraperitoneally.



of body weight, R and S rats in the cadmium and control groups (n=40) had identical kidney and liver weights which were 0.4 ± 0.06 and 3.9 ± 0.4 g, respectively.

3. Cadmium Concentrations in Tissues.

Figure 9 shows that the concentrations of cadmium in the kidney and liver tissues of S rats (4 males and 6 females) given cadmium were significantly higher ($p < 0.001$) than that of R rats (4 males and 6 females). Since no significant difference ($p > 0.05$) appeared between tissues obtained from male and female rats, these values were pooled. Cadmium levels in renal and hepatic tissues in saline-injected controls (4 males and 6 females of either strain) were 0.72 ± 0.12 and 0.25 ± 0.05 (R rats); 0.81 ± 0.23 and 0.30 ± 0.04 (S rats) $\mu\text{g/g}$ wet weight, respectively.

4. Renal Vascular Changes in Response to Cadmium Injection.

Histological kidney sections were evaluated without knowledge of strain and group. Microscopic changes of glomeruli (thickening of capillary basement membrane; hyaline deposits along the basement membrane; increase in number of epithelial cells, hyalinization and atrophy) arterioles and arteries (hyperplasia of intima; hypertrophy of media; sclerosis; narrowing of the lumen; necrotizing lesions) were graded as follows: normal = 0, slight = +, moderate = ++ and marked = +++. Since no significant ($p > 0.05$) renal vascular changes were noted in the male R and S cadmium rats and their respective controls, these data were not presented. A summary of the renal vascular changes in female R and S rats is shown in Table 2. Although changes occurred

FIGURE 9

Cadmium concentrations in the kidney and liver of R and S rats injected with cadmium (1 and 2 mg/kg, intraperitoneally). Tissues of 10 animals (4 males, 6 females) of each strain were analyzed for cadmium, pooling left and right renal tissues. Each bar represents the mean $\pm$ SEM.

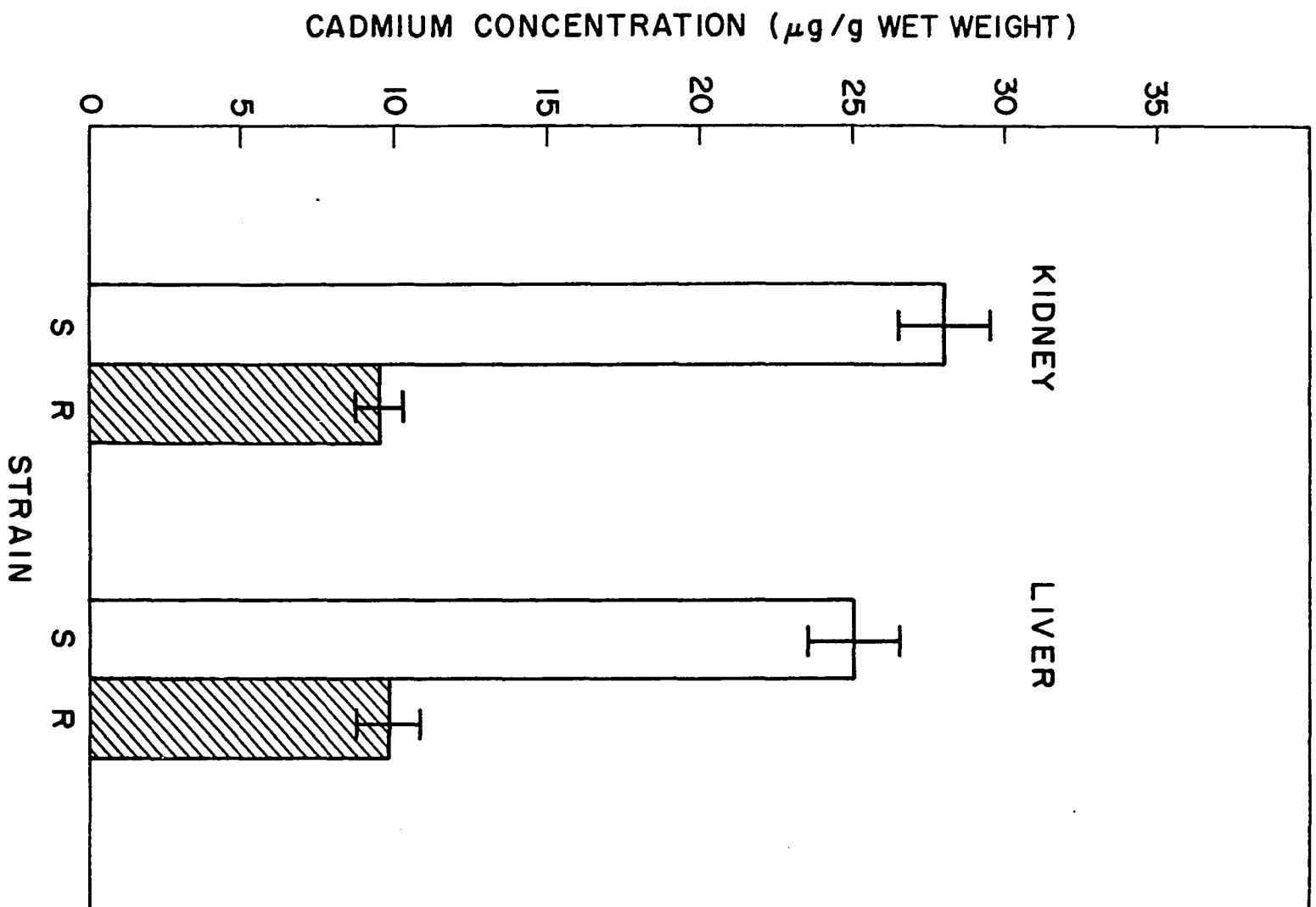


**TABLE 2. RENAL VASCULAR CHANGES IN FEMALE R AND S RATS INJECTED
WITH CADMIUM**

GROUP	GLOMERULI		ARTERIOLES		ARTERIES	
	0	+ -++	0	+ -++	0	+ -++
CONTROL						
R (10) [*]	10	0	10	0	10	0
S (10)	8	2	7	3	9	1
CADMIUM						
R (10)	8	2	7	3	8	2
S [†] (10)	1	9	2	8	4	6

0 = Normal.

+ -++ = Slight to moderate changes.

* = Number of rats examined.

† = Differs from any other group ($p < 0.01$).

to some extent in all the groups except in the R controls, the S cadmium group exhibited significantly more changes than any other group ($p < 0.01$).

C. CHRONIC CADMIUM STUDY 2:

1. Effects of Cadmium Injection on Blood Pressure and Body Weight.

The pertinent blood pressure and body weight data are shown in Table 3. The anticipated difference in blood pressure between R and S controls ($p < 0.001$) was evident. But the main objective of this study was to observe the differences in blood pressure between cadmium-injected rats and saline-injected controls within each strain. Among R and S rats in the cadmium-injected group, blood pressure, as compared with their respective controls, was not significantly changed ($p > 0.05$) 3 weeks after the first intraperitoneal (IP) cadmium (2 mg/kg) injection (week 6). Following a second dose of cadmium (1 mg/kg, IP) on week 6, S rats developed significantly higher blood pressure than their respective controls ($p < 0.001$). R rats, by contrast, remained normotensive throughout the study. Body weights of R and S rats in the cadmium group and their corresponding controls were not statistically significant ($p > 0.05$). Moreover, in terms of percent of body weight, R and S cadmium and control rats ($n=24$) had similar kidney and liver weights which were 0.38 ± 0.09 and 4.2 ± 0.7 g, respectively. All the animals were sacrificed at the onset of cadmium-induced hypertension in S rats on week 8.

**TABLE 3. EFFECTS OF CADMIUM INJECTION ON BLOOD PRESSURE AND BODY WEIGHT
IN FEMALE R AND S RATS**

GROUP	AGE 6 WEEKS		AGE 8 WEEKS	
	BP (mm Hg)	BW (g)	BP (mm Hg)	BW (g)
CADMIUM				
R (6)*	118.3 $\pm$ 4.3 $\square$	146.3 $\pm$ 5.6	121.2 $\pm$ 4.7	173.4 $\pm$ 4.7
S (6)	142.3 $\pm$ 3.2	150.0 $\pm$ 5.5	167.5 $\pm$ 3.0 Δ	170.0 $\pm$ 5.2
CONTROL				
R (6)	115.1 $\pm$ 3.1	148.2 $\pm$ 5.4	125.3 $\pm$ 3.1	175.0 $\pm$ 4.3
S (6)	142.0 $\pm$ 2.2	148.1 $\pm$ 6.5	150.0 $\pm$ 2.8	172.1 $\pm$ 5.4

BP = Systolic blood pressure.

BW = Body weight.

* = Number of rats.

$\square$ = Mean $\pm$ SEM.

Δ = Differs from the S control group ($p < 0.001$).

2. Cadmium Levels in the Renal Cortex and Medulla and Liver at the Onset of Cadmium-Induced Hypertension.

The data are illustrated in Figure 10. The levels of cadmium in the renal cortex and medulla and liver of S rats (n=6) were significantly higher than that of R rats (n=6) at the onset of cadmium-induced hypertension on week 8. Cortical, medullary and hepatic cadmium levels in R and S rats in the control group were 0.28 ± 0.04 , 0.07 ± 0.03 and 0.07 ± 0.02 $\mu\text{g/g}$ wet weight, respectively. Since no significant difference ($p > 0.05$) appeared between tissues obtained from R and S controls, these values were pooled.

D. CHRONIC CADMIUM STUDY 3:

1. Effect of Cadmium Injection on Blood Pressure.

The data are represented in Figures 11 and 12. An analysis of variance indicated: (1) adult female rats in the cadmium group manifested higher elevation of blood pressure ($p < 0.01$) than adult male rats in the same group; and (2) the anticipated strain difference with S controls exhibiting significantly higher blood pressure than R controls ($p < 0.001$). But the goal of this study was to investigate the differences in blood pressure between cadmium-injected rats and saline-injected controls within each strain. Among animals exposed to cadmium (Cd), blood pressure, as compared with appropriate controls, was not significantly affected ($p > 0.05$) 3 weeks after the first intraperitoneal (IP) cadmium (2 mg/kg) injection (week 19). Following a second dose of cadmium (1 mg/kg, IP) on week 19, male and female S rats developed significantly higher ($p < 0.001$) blood pressure (162.1 ± 4.2 and $170 \pm 4 \pm 5.2$ mmHg, respectively), than their respective controls

FIGURE 10

Cadmium concentrations in the renal cortex and medulla and liver of female R and S rats at the onset of cadmium-induced hypertension. Tissues of 12 animals of either strain were assayed for cadmium, pooling left and right kidney tissues. Each bar represents the mean $\pm$ SEM. The animals were exposed to cadmium (1 and 2 mg/kg) by intraperitoneal injection.

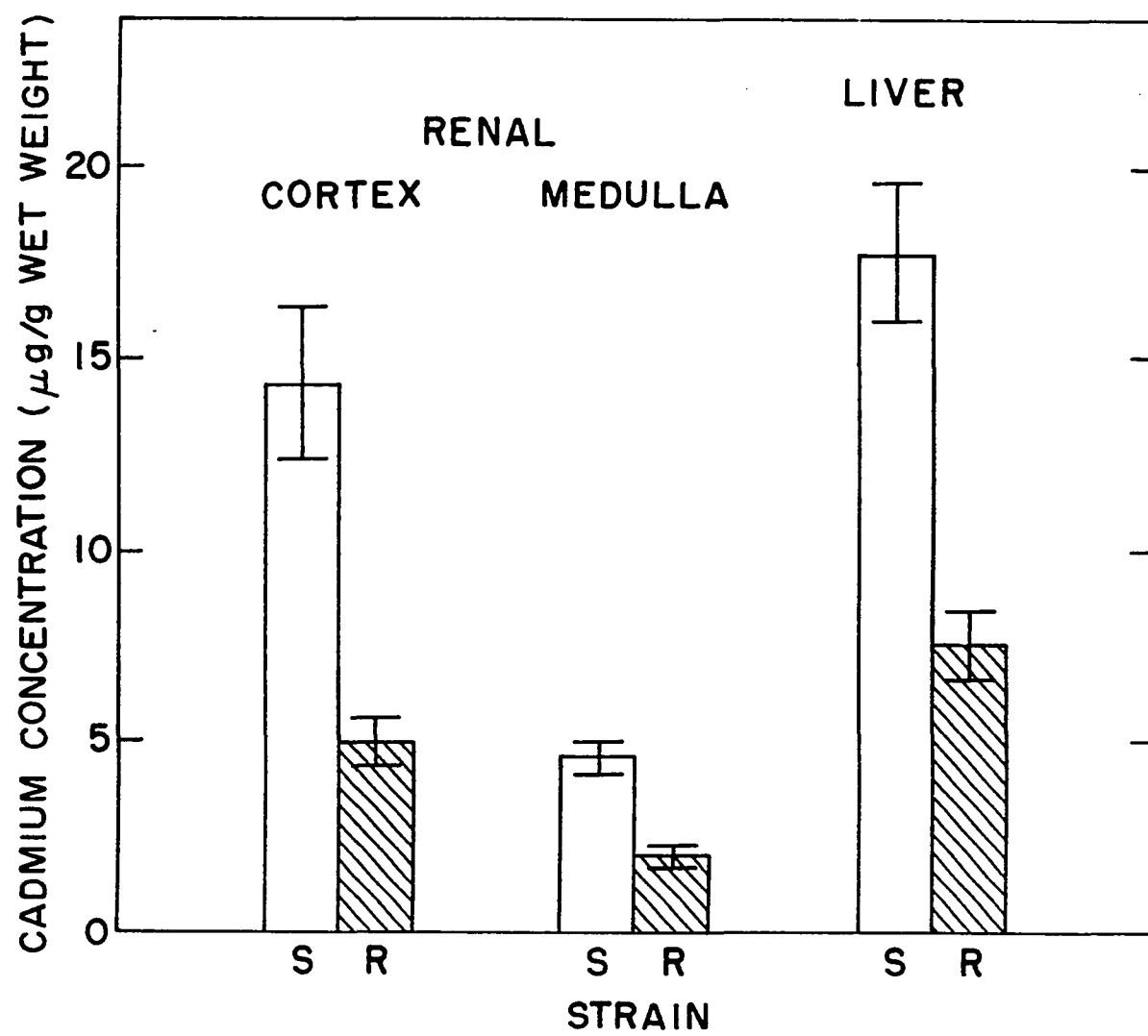


FIGURE 11

Effect of cadmium injection on blood pressure in adult male R and S rats. Each symbol represents the mean of 5 rats. R and S cadmium rats were given cadmium (2 mg/kg) on week 16 followed by second and third injections of cadmium (1 mg/kg) on weeks 19 and 40. R rats received an additional injection of (1 mg/kg) on week 35. R and S controls were given saline injections. All injections were given intraperitoneally.

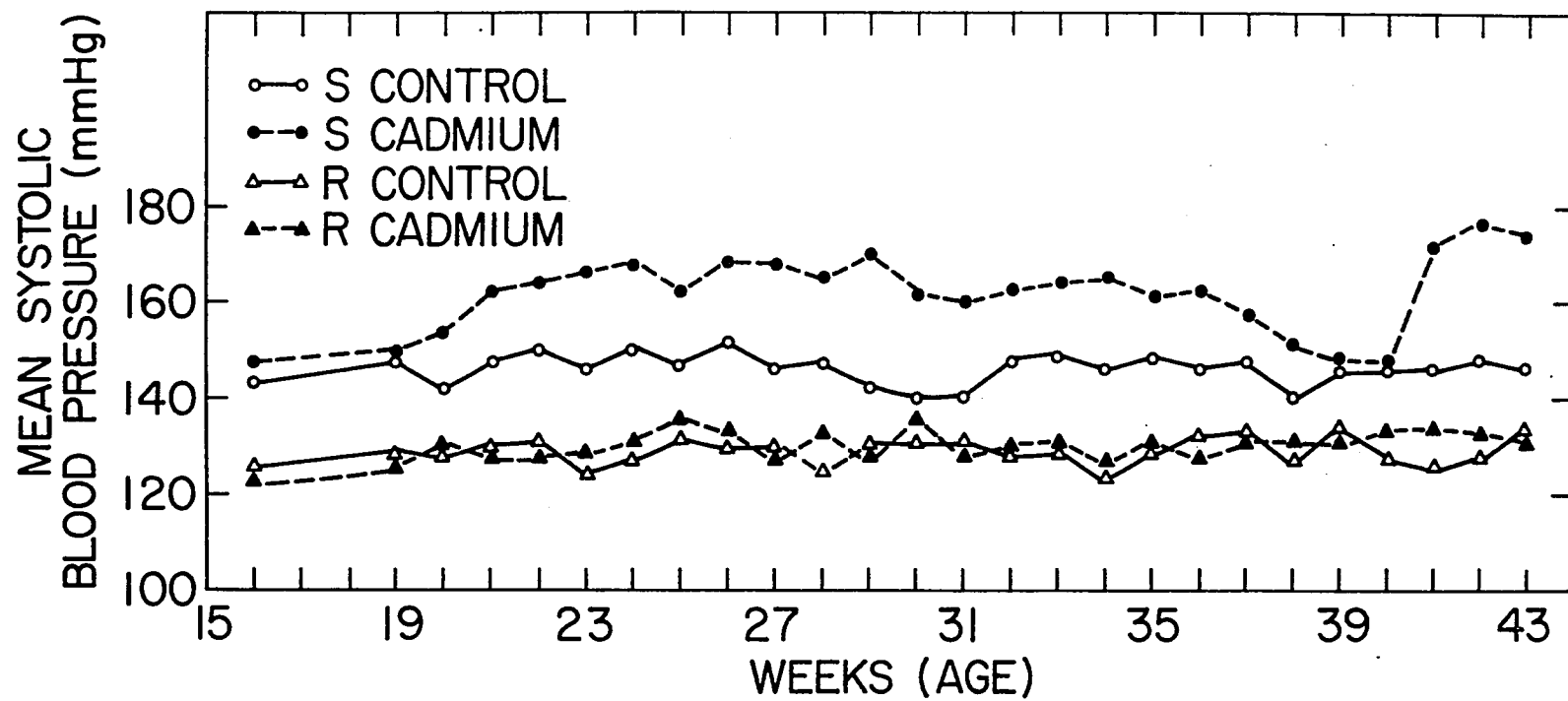
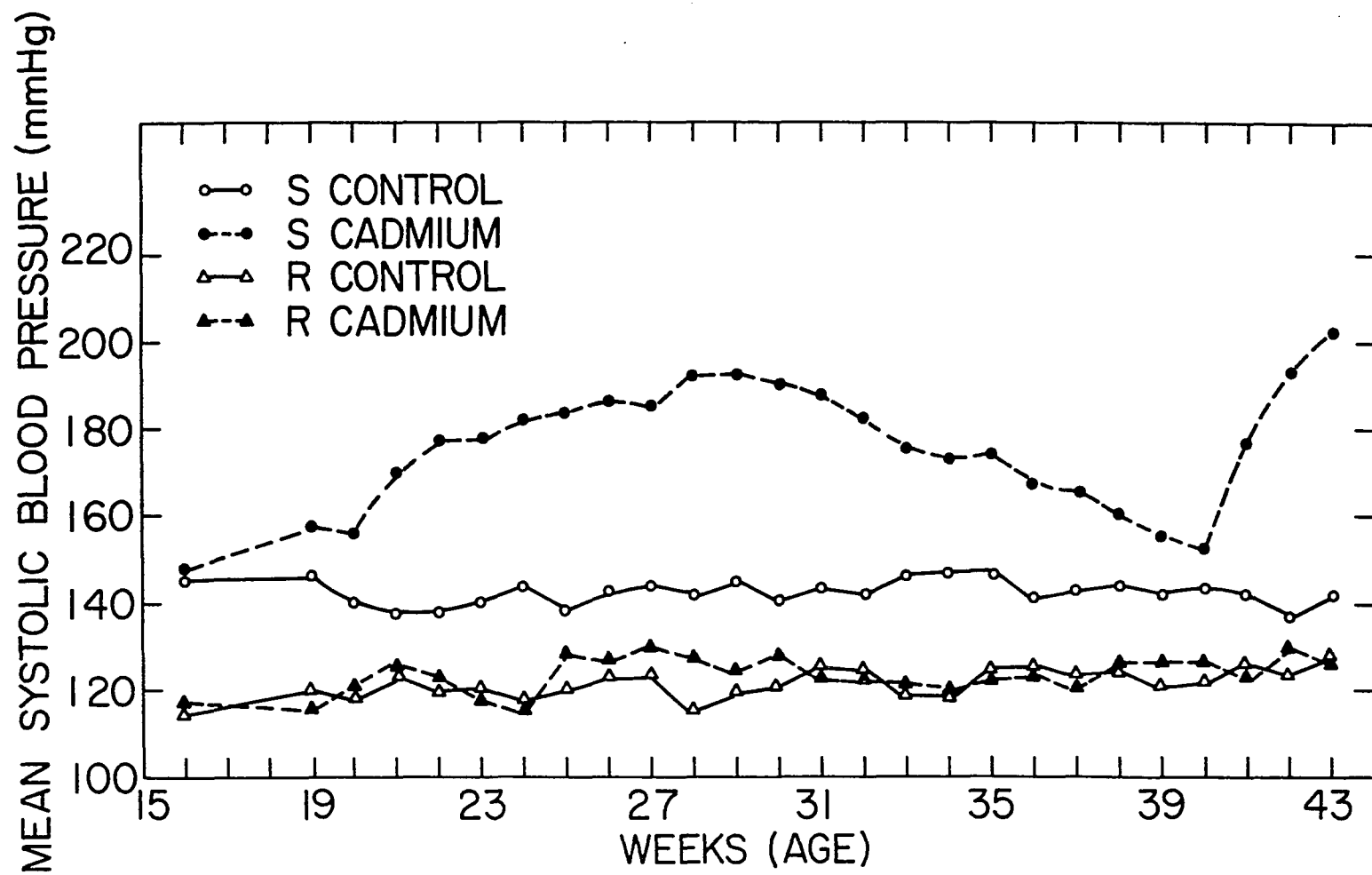


FIGURE 12

Effect of cadmium injection on blood pressure in adult female R and S rats. Each symbol represents the mean systolic blood pressure of 5 animals. R and S cadmium rats were injected with cadmium (2 mg/kg) on week 16 and followed by second and third injections of cadmium (1 mg/kg) on weeks 19 and 40. R rats received an additional injection of cadmium (1 mg/kg) on week 35. R and S controls were given saline injections. All injections were administered intraperitoneally.



(148.4 ± 4.3 and 137.2 ± 9.1 mmHg, respectively) on week 21. The cadmium-induced hypertension continued through weeks 38 (S males) and 39 (S females), after which the difference in blood pressure between the cadmium-injected rats and their corresponding controls were not statistically significant ($p > 0.05$). In response to a third injection of 1 mg Cd/kg given to rats intraperitoneally on week 40, the difference in blood pressure between S cadmium males and females (171.6 ± 4.8 and 176.4 ± 5.6 mmHg, respectively) and their respective controls (146.1 ± 4.4 and 142.4 ± 4.0 mmHg, respectively), once again achieved statistical significance ($p < 0.001$) by week 41 and continued thereafter. Despite an additional injection of cadmium (1 mg/kg, IP) on week 35, R rats remained normotensive throughout the experiment. None of the controls developed hypertension. Morbidity and mortality commenced among cadmium-injected males and females of either strain to the same extent on week 43 and there were no healthy survivors to make a significant comparison thereafter. Of those rats examined post mortem, 80% of the rats in the cadmium group died of massive internal hemorrhage.

2. Effect of Cadmium Injection on Body Weight.

The data are demonstrated in Table 4. An analysis of body weight showed the expected significant difference between adult males and females of either strain ($p < 0.001$). However, weights of males and females of both strains in the cadmium group and their corresponding controls were not statistically different ($p > 0.05$). In addition, in terms of percent of body weight, R and S rats (5 male and 5 female) in both cadmium and control groups ($n = 40$) had similar kidney and

TABLE 4.

EFFECT OF CADMIUM INJECTION ON BODY WEIGHT IN MALE AND FEMALE R AND S RATS

SEX	GROUP	BODY WEIGHT (g)							
		WEEKS (AGE)							
		16	19	23	27	31	35	39	43
MALE	CONTROL								
	R (5)*	370.0 $\pm$ 4.0 $\square$	384.8 $\pm$ 7.8	408.4 $\pm$ 5.2	429.0 $\pm$ 7.3	450.8 $\pm$ 4.2	459.2 $\pm$ 4.3	464.4 $\pm$ 8.0	468.1 $\pm$ 5.0
	S (5)	369.8 $\pm$ 3.4	379.3 $\pm$ 8.4	403.9 $\pm$ 7.3	427.5 $\pm$ 8.1	441.3 $\pm$ 6.5	458.0 $\pm$ 5.5	461.8 $\pm$ 7.4	468.1 $\pm$ 6.3
	CADMIUM								
	R (5)	371.2 $\pm$ 5.0	380.8 $\pm$ 17.3	411.4 $\pm$ 7.3	436.4 $\pm$ 9.3	448.5 $\pm$ 6.2	457.8 $\pm$ 6.3	461.7 $\pm$ 7.0	474.6 $\pm$ 6.5
	S (5)	365.6 $\pm$ 4.5	373.6 $\pm$ 10.5	410.7 $\pm$ 6.4	431.8 $\pm$ 7.1	448.2 $\pm$ 5.2	461.7 $\pm$ 5.1	466.3 $\pm$ 6.4	471.9 $\pm$ 4.1
FEMALE	CONTROL								
	R (5)	260.7 $\pm$ 5.0	265.3 $\pm$ 4.5	278.3 $\pm$ 4.0	284.3 $\pm$ 5.5	288.4 $\pm$ 4.0	296.5 $\pm$ 4.3	293.5 $\pm$ 4.5	298.5 $\pm$ 6.0
	S (5)	254.8 $\pm$ 4.0	260.0 $\pm$ 17.5	272.0 $\pm$ 4.3	280.5 $\pm$ 5.0	284.3 $\pm$ 6.1	290.3 $\pm$ 5.3	289.2 $\pm$ 5.0	294.2 $\pm$ 4.3
	CADMIUM								
	R (5)	247.2 $\pm$ 3.8	250.8 $\pm$ 15.2	263.8 $\pm$ 4.0	275.2 $\pm$ 4.3	287.4 $\pm$ 5.1	289.5 $\pm$ 4.2	296.4 $\pm$ 5.3	305.3 $\pm$ 3.3
	S (5)	249.4 $\pm$ 4.3	253.7 $\pm$ 15.0	269.2 $\pm$ 6.3	275.0 $\pm$ 6.2	290.1 $\pm$ 3.4	296.3 $\pm$ 4.3	293.4 $\pm$ 4.4	301.4 $\pm$ 5.5

* = Number of rats.

 $\square$ = Mean $\pm$ SEM.

liver weights which were 0.37 ± 0.04 and 4.0 ± 0.8 g, respectively.

3. Cadmium Concentrations in Tissues.

The data are illustrated in Figure 13. The concentrations of cadmium in the left and right kidneys and liver of S rats (5 male and 5 female) given cadmium were significantly higher ($p < 0.001$) than that of R rats (5 male and 5 female). Renal and hepatic cadmium levels in R and S rats in the control group were 0.83 ± 0.24 and 0.52 ± 0.12 $\mu\text{g/g}$ wet weight, respectively. Since no significant difference ($p > 0.05$) appeared between tissues obtained from males (5 R and 5 S) and females (5 R and 5 S), these values were pooled.

4. Renal Vascular and Hepatic Changes in Response to Cadmium Injection.

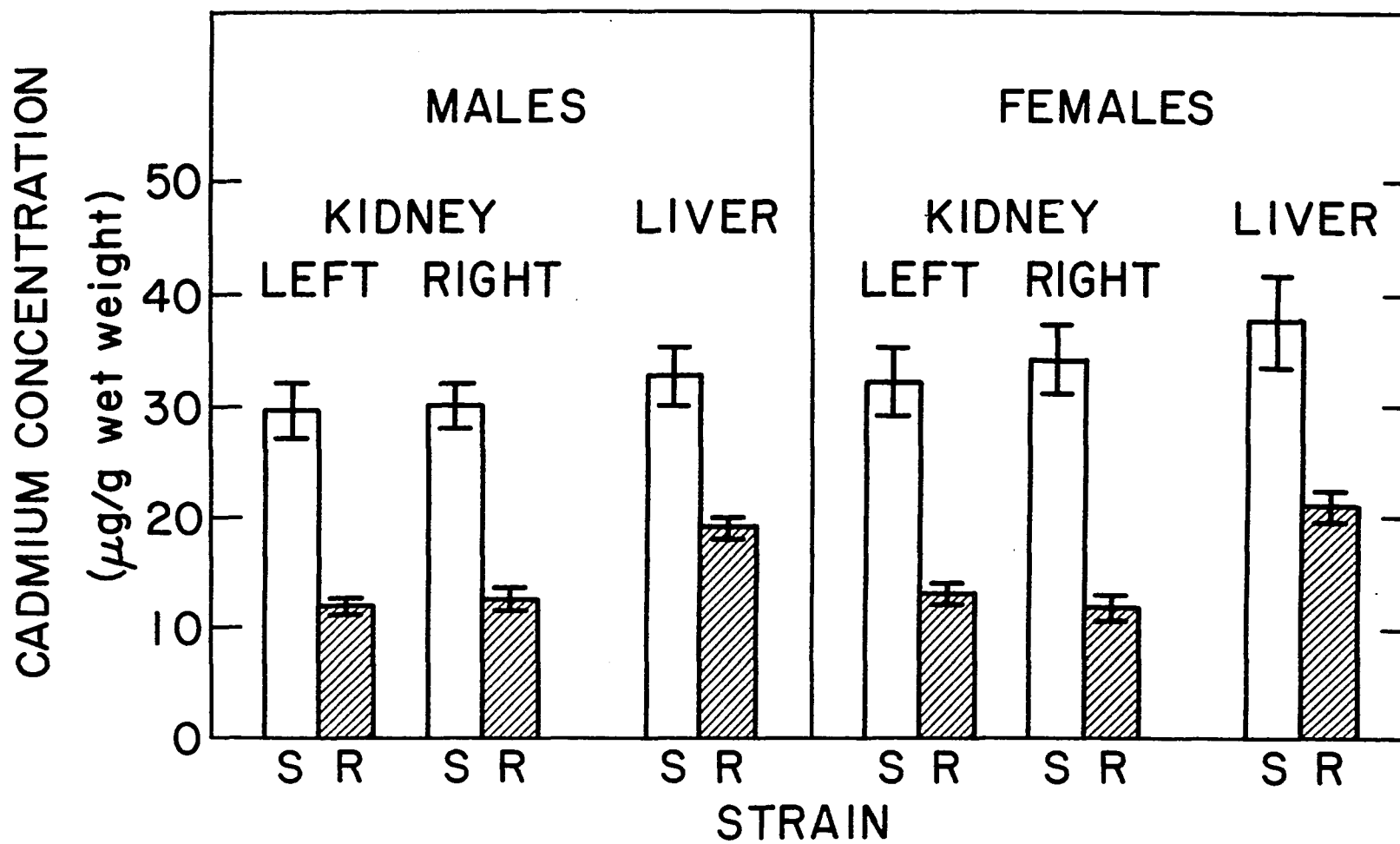
(a) Kidney: Microscopic changes of glomeruli (thickening of capillary basement membrane; hyaline deposits along the basement membrane; increase in number of epithelial cells; hyalinization and atrophy) arterioles and arteries (hyperplasia of intima; hypertrophy of media; sclerosis; narrowing of the lumen; necrotizing lesions) were graded as follows: normal = 0, slight = +, moderate = ++ and marked = +++.

(b) Liver: Hepatic morphological changes (cirrhosis, fatty degeneration and vacuolation of cells) were graded as follows: normal = 0, slight = +, moderate = ++ and marked = +++.

Since no significant ($p > 0.05$) renal vascular and hepatic changes were observed between males and females of either strain in both cadmium and control groups, these values were pooled. A summary of the renal vascular

FIGURE 13

Cadmium concentrations in the kidneys and liver of R and S rats exposed to cadmium by injection (1 and 2 mg/kg, intraperitoneally). Tissues of 5 animals were assayed for cadmium and each bar represents the mean $\pm$ SEM.



changes in R and S rats of either sex is shown in Table 5. Although changes occurred to some extent in all the groups, the S cadmium group exhibited significantly more changes than any other group ($p < 0.01$).

E. CHRONIC CADMIUM STUDY 4:

1. Effect of Cadmium Injection on the Course of Salt-Induced Hypertension.

The pertinent blood pressure data are summarized in Figure 14. Among female S animals in the cadmium (Cd) group and fed rat food containing 4% NaCl, blood pressure, as compared with the corresponding saline-injected S controls, was not significantly affected 3 weeks (week 6) after the administration of 2 mg Cd/kg intraperitoneally (IP). Following a second dose of cadmium (1 mg/kg, IP) on week 6, S cadmium rats developed significantly higher ($p < 0.01$) blood pressure (166.3 ± 6.2 mmHg) than their respective controls (158.2 ± 6.8 mmHg) by week 7. This difference in blood pressure between the two groups lasted through week 14, a total of 8 weeks. In response to a third injection of cadmium (1 mg/kg, IP) given on week 23, the difference once again in blood pressure achieved statistical significance ($p < 0.01$) by week 24. This latter injection of cadmium produced a fulminating elevation of blood pressure (245.7 ± 16.2 mmHg) and accelerated the morbidity and mortality of salt-induced hypertension to an extent that no survivors were found in the S cadmium group (originally 10 rats) by week 25. Conversely, the 10 S rats in the control group exposed to salt insult alone were all alive and apparently healthy on week 25. Of those S cadmium rats examined post mortem, internal hemorrhage was the major

**TABLE 5. RENAL VASCULAR AND HEPATIC CHANGES IN ADULT MALE AND FEMALE
R AND S RATS INJECTED WITH CADMIUM**

GROUP	KIDNEY						LIVER		
	GLOMERULI		ARTERIOLES		ARTERIES				
	0	+--+	0	+--+	0	+--+	0	+--+	+++
CONTROL									
R (10) [*]	8	2	9	1	9	1	8	2	0
S (10)	6	4	8	2	7	3	8	2	0
CADMIUM									
R (10)	7	3	8	2	8	2	2	8	0
S [†] (10)	1	9	2	8	2	8	0	2	8

* = Number of rats (5 male and 5 female) examined.

0 = Normal.

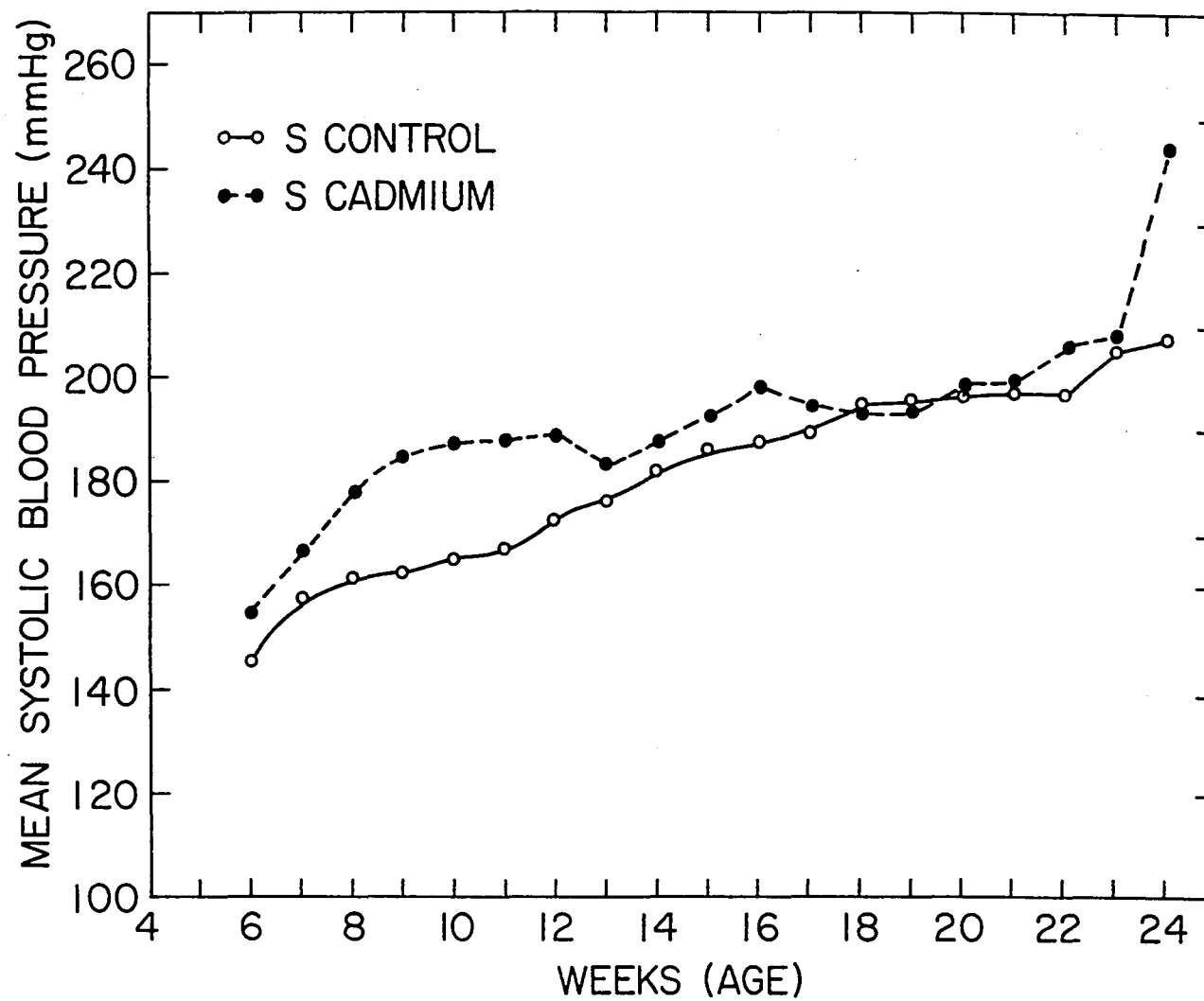
+--+ = Slight to moderate changes.

+++ = Marked changes.

† = Differs from any other group ($p < 0.01$).

FIGURE 14

Effect of cadmium injection on salt-induced hypertension in female S rats. Each symbol represents the mean of 10 animals. S cadmium rats were injected with cadmium (2 mg/kg) on week 3 and followed by second and third injections of cadmium (1 mg/kg) on weeks 6 and 23. S controls were given saline injections. All injections were administered intraperitoneally.



cause of death. In addition, 20% of animals in each group showed incidence of polyarteritis nodosa. Grossly, the lesions were detected in the mesenteric arteries. They usually formed tortuous, adherent, nodular masses.

2. Effect of Cadmium Injection on Body Weight.

The data are presented in Table 6. An analysis of variance did not indicate any significant difference ($p > 0.05$) in body weight between female S rats in the cadmium group and their respective saline-injected controls ($p > 0.05$).

3. Renal Vascular Changes in Response to Cadmium Injection.

Microscopic changes of glomeruli (thickening of capillary basement membrane; hyaline deposits along the basement membrane; increase in number of epithelial cells; hyalinization and atrophy) arterioles and arteries (hyperplasia of intima; hypertrophy of media; sclerosis; narrowing of the lumen; necrotizing lesions) were graded as follows: normal = 0, slight = +, moderate = ++ and marked = +++. Table 7 shows a summary of the renal vascular changes in female S rats in the control and cadmium groups. Moderate to marked morphological changes observed in both S control and cadmium groups were not statistically significant ($p > 0.05$).

CHRONIC CADMIUM STUDY 5:

1. Effect of Cadmium Injection on the Course of Two-Kidney Goldblatt Hypertension.

The data are illustrated in Figure 15. The anticipated strain difference was observed with male S rats exhibiting significantly higher ($p < 0.001$) blood pressure than male R rats prior to subject-

TABLE 6. EFFECT OF CADMIUM INJECTION ON BODY WEIGHT IN FEMALE S RATS
ON HIGH SALT DIET

GROUP (N)	BODY WEIGHT (g)			
	WEEKS (AGE)			
	6	12	18	24
S CONTROL (10)	149.4 $\pm$ 4.1 $\square$	226.8 $\pm$ 3.1	257.1 $\pm$ 6.2	271.4 $\pm$ 4.2
S CADMIUM (10)	150.1 $\pm$ 5.3	226.2 $\pm$ 3.5	260.3 $\pm$ 5.3	272.4 $\pm$ 3.1

N = Number of rats.

$\square$ = Mean $\pm$ SEM.

TABLE 7. RENAL VASCULAR CHANGES IN FEMALE S RATS FED HIGH SALT DIET
AND INJECTED WITH CADMIUM

GROUP (N)	GLOMERULI		ARTERIOLES		ARTERIES	
	++	+++	++	+++	++	+++
S CONTROL (10)	4	6	3	7	5	5
S CADMIUM (10)	2	8	4	6	4	6

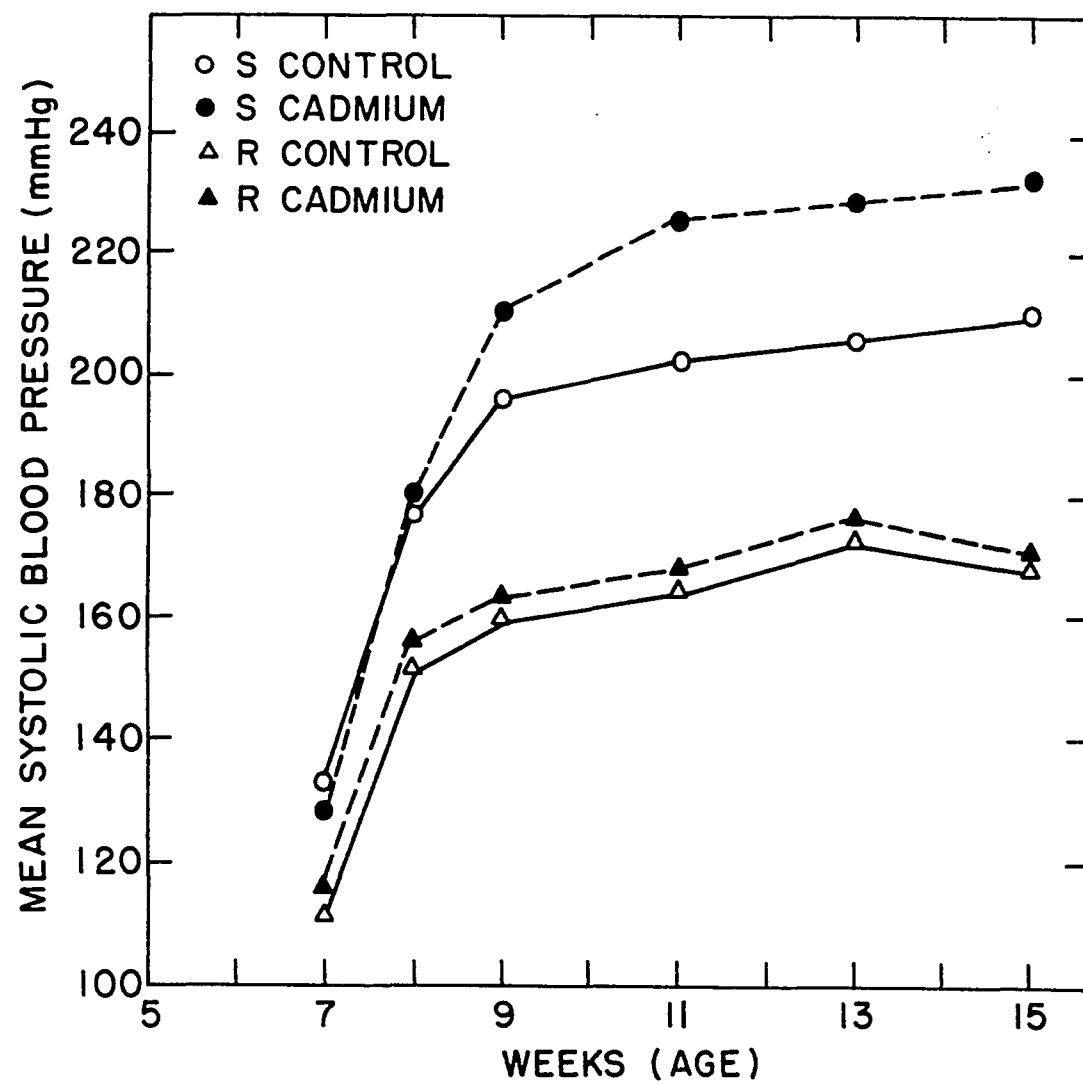
N = Number of rats examined.

++ = Moderate changes.

+++ = Marked changes.

FIGURE 15

Effect of cadmium injection on two-kidney Goldblatt hypertension in male R and S rats. Each symbol represents the mean $\pm$ SEM of 6 animals. All the animals had clipped right renal artery. Following the clipping, cadmium and control rats received intraperitoneal injections of cadmium (2 mg/kg) and saline, respectively.



ing both strains to constriction of the right renal artery on week 7. But the main objective of this study was to observe the differences in blood pressure between cadmium-injected rats and saline-injected controls within each strain.

Following the operation and intraperitoneal (IP) cadmium (2 mg/kg) administration on week 7, the S cadmium group (6 rats) exhibited marked elevation of blood pressure (210.6 ± 4.2 mmHg) which was significantly higher ($p < 0.01$) than their corresponding controls (6 rats) who averaged 196.6 ± 5.3 mmHg by week 8. This difference in blood pressure continued through week 15 after which morbidity and mortality started among S cadmium rats and there were no healthy subjects to make a significant comparison by week 16. Hence, the study was terminated. No significant difference ($p > 0.05$) in blood pressure was noticed between the R cadmium (6 rats) and their respective controls (6 rats). Morbidity and mortality were not observed in S rats in the control group and in R rats in both cadmium and control groups on week 16. At necropsy, internal hemorrhage was not apparent in R and S rats in the cadmium group.

2. Effect of Cadmium Injection on Body Weight.

Body weights of male R and S rats in the cadmium group and their corresponding controls did not differ statistically ($p > 0.05$) as shown in Table 8. Comparing the measured weights of left (intact) and right (clipped) kidneys at necropsy, R and S rats in the cadmium and control groups showed reduction of renal mass in the right kidney which was indicated by mean weights of 24-29% of those of the left. In terms of percent of body weight, weights of the left and right

**TABLE 8. EFFECT OF CADMIUM INJECTION ON BODY WEIGHT IN MALE R AND S RATS
WITH RENAL ARTERY CONSTRICTION**

GROUP	BODY WEIGHT (g)		
	WEEKS (AGE)		
	7	11	15
CONTROL			
R (6)*	222.4 $\pm$ 5.0	310.4 $\pm$ 5.4	384.3 $\pm$ 6.0
S (6)	226.6 $\pm$ 4.1	315.6 $\pm$ 7.2	380.1 $\pm$ 5.1
CADMIUM			
R (6)	220.5 $\pm$ 4.3	312.2 $\pm$ 5.1	379.3 $\pm$ 6.2
S (6)	225.5 $\pm$ 3.2	310.6 $\pm$ 6.2	383.8 $\pm$ 7.5

* = Number of rats.

$\square$ = Mean $\pm$ SEM.

kidneys, and liver were 0.43 ± 0.03 , 0.34 ± 0.02 and 3.8 ± 0.7 g, respectively. Since no significant difference ($p > 0.05$) was found between weights obtained from the cadmium (6 R and 6 S) and control (6 R and 6 S) groups, these values were pooled.

3. Cadmium Concentrations in Tissues.

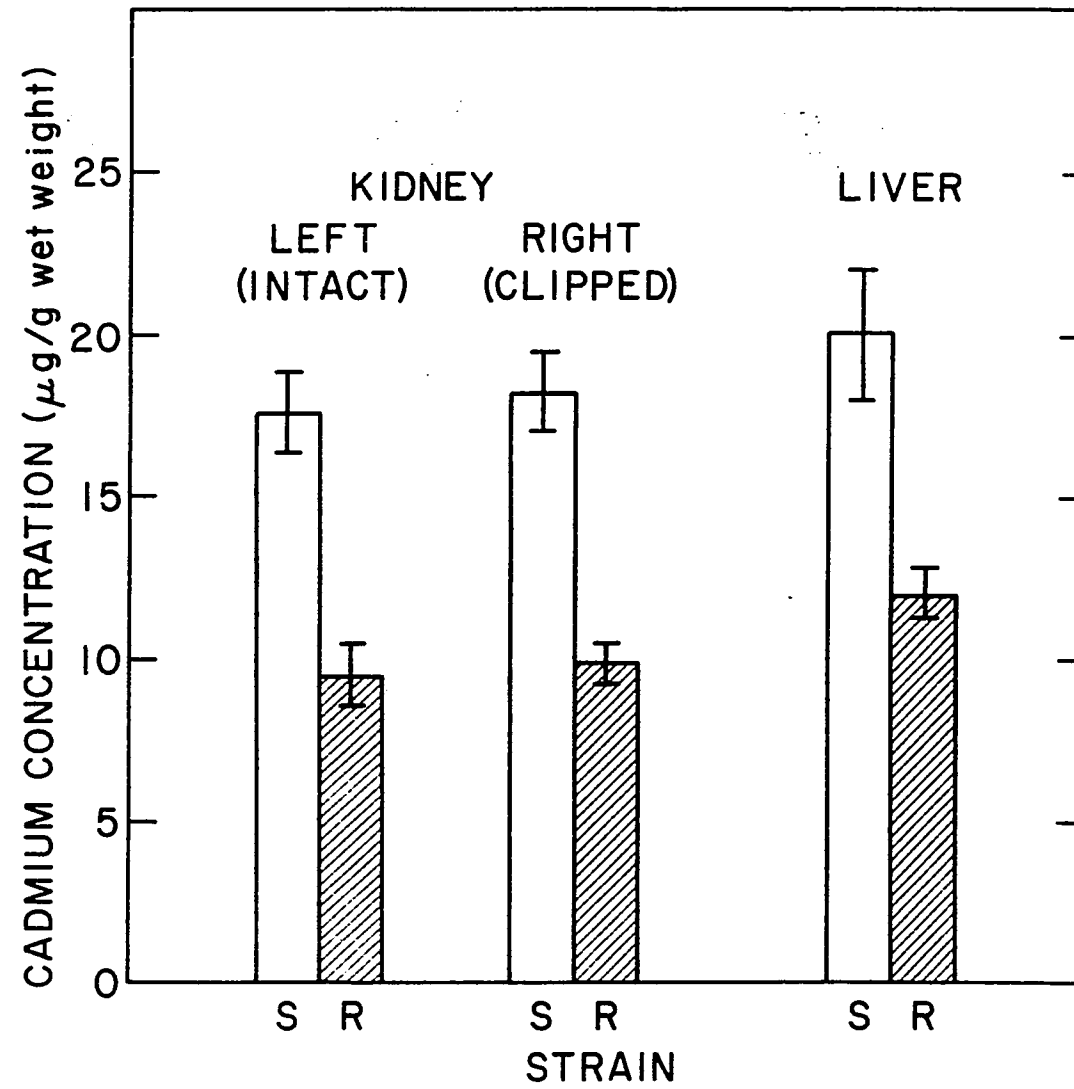
Figure 16 shows that cadmium levels in left (intact) and right (clipped) renal tissues were comparable in male R and S rats injected with cadmium despite the smaller size of the right kidney. Therefore, constriction of the right renal artery resulted in no loss or gain of cadmium in the affected kidney. Furthermore, the concentrations of cadmium in both kidneys and liver tissues in S rats given cadmium were significantly higher ($p < 0.001$) than that of R rats. Renal and hepatic cadmium levels in R and S rats in saline-injected controls (6 R and 6 S) were 0.48 ± 0.21 and 0.18 ± 0.06 $\mu\text{g/g}$ wet weight, respectively. Since no significant difference ($p > 0.05$) appeared between tissues obtained from R and S rats, these values were combined.

4. Renal Vascular Changes in Response to Cadmium Injection.

(a) Right (clipped) kidney: Male R and S rats in the cadmium and control groups showed similar morphological changes in the clipped right kidney as follows: slight to moderate diffuse tubular atrophy, partial infarction of the kidney, slight narrowing of the cortex with reduction in size of the glomeruli in the subcapsular zone. Acute renal vascular changes such as occurred in the counterlateral left (intact) kidney were not observed. Since the changes in the right kidney were not significant ($p > 0.05$) between R and S rats in the

FIGURE 16

Cadmium concentrations in the kidneys and liver of male R and S rats with renal hypertension. Tissues of 6 animals of either strain were assayed for cadmium and each bar represents the mean $\pm$ SEM. The animals were given cadmium (2 mg/kg) intraperitoneally.



cadmium group and their respective controls, these data were not presented.

(b) Left (intact) kidney: (Table 9). Changes of glomeruli (thickening of capillary basement membrane; hyaline deposits along the basement membrane, increase in number of epithelial; hyalinization and atrophy) arterioles and arteries (hyperplasia of intima; hypertrophy of media; sclerosis; narrowing of the lumen; necrotizing lesions) were graded as follows: normal = 0, slight = +, moderate = ++ and marked = +++. Since no significant ($p > 0.05$) renal vascular changes were detected between cadmium-injected R and S rats and their respective saline-injected controls, these values were combined. Although changes occurred in both R and S rats, S rats exhibited significantly more changes ($p < 0.01$) than their counterpart R rats.

G. CHRONIC CADMIUM STUDY 6:

1. Intake of Cadmium in Water.

There was no difference in water intake between female R and S rats within a group. For the control (0 mg Cd/L) and cadmium-fed (1 to 5 mg/L) groups, the average intake for either strain on low salt (0.4% NaCl) diet was approximately 15 ml per rat per day whereas a rat in the group receiving 10 mg Cd/L drank approximately 10 ml daily. Furthermore, rats fed high salt (4% NaCl) diet and ingesting cadmium (0 to 10 mg/L) drank one and half times as much as the corresponding rats on low salt diet. Table 10 summarizes the amount of cadmium ingested in water at 4, 12, 20 and 28 weeks of cadmium exposure. Water consumption was used as an index of cadmium intake

**TABLE 9. RENAL VASCULAR CHANGES IN CADMIUM-INJECTED MALE R AND S RATS
WITH RENAL HYPERTENSION**

STRAIN [*]	GLOMERULI			ARTERIOLES			ARTERIES		
	0	+--+	+++	0	+--+	+++	0	+--+	+++
R (12) [#]	4	8	0	6	6	0	8	4	0
S [□] (12)	0	4	8	0	6	6	0	7	5

* Since no significant renal vascular changes were observed between R and S rats injected with cadmium and their respective saline-injected controls, these values were pooled.

= Number of rats.

0 = Normal.

+--+ = Slight to moderate changes.

+++ = Marked changes.

□ = $p \leq 0.01$ compared with the R strain.

TABLE 10. CADMIUM INTAKE OF FEMALE R AND S RATS EXPOSED TO 1, 2.5, 5 and 10 mg CADMIUM/L DRINKING WATER*

DIET	CADMIUM IN DRINKING WATER (mg/L)	CADMIUM INTAKE (μ g/kg/day)							
		WEEKS (POSTWEANING)							
		4		12		20		28	
		R	S	R	S	R	S	R	S
LOW SALT (0.4% NaCl)	1	87	88	69	67	62	61	58	57
	2.5	224	222	175	171	159	155	151	150
	5	463	470	349	337	314	304	309	303
	10	657	658	476	478	402	395	441	433
HIGH SALT (4% NaCl)	1	135	132	104	102	93	90	91	89
	2.5	340	336	258	260	227	230	225	229
	5	673	656	519	508	458	444	450	441
	10	967	974	717	702	581	561	629	647

*Each group of rats (10 R and 10 S) were weighed as a group and the volume of water consumed by each group was noted as an index of cadmium intake.

which depended on the concentration of cadmium in the drinking water and salt content in the rat food.

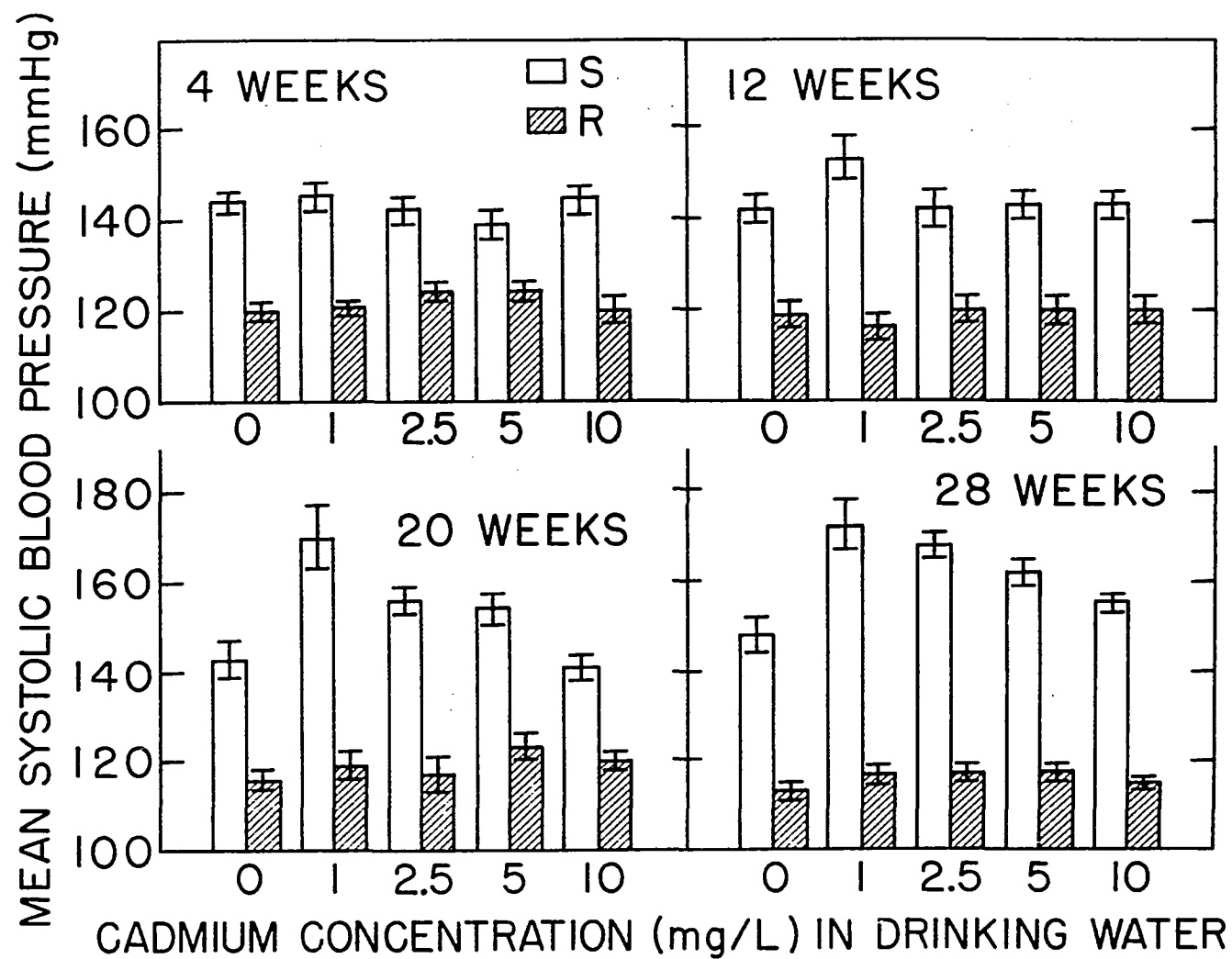
2. Effect of Cadmium Feeding on Blood Pressure.

(a) Low Salt Diet (Figure 17). An analysis of variance revealed the anticipated difference in blood pressure between female R and S rats in the control group ($p < 0.001$). But the main goal of this study was to observe the differences in blood pressure between cadmium-fed rats and controls within each strain. Among R and S rats in cadmium-fed groups (1 to 10 mg/L), blood pressure, as compared with their respective controls, was not significantly changed ($p > 0.05$) after 4 weeks of cadmium (Cd) exposure. At week 12, S rats exposed to the lowest dose of cadmium (1 mg/L) exhibited significantly higher ($p < 0.01$) elevation of blood pressure than the controls. After both 20 and 28 weeks of exposure, S rats receiving 1 to 5 mg Cd/L had significant increases ($p < 0.01$) in systolic blood pressure. By contrast, the difference between the means of S rats ingesting the highest dose of cadmium (10 mg/L) and the controls (0 mg Cd/L) was without statistical significance ($p > 0.05$). R rats remained normotensive regardless of cadmium feeding throughout the study.

At week 29, weight loss and mortality from bronchopneumonia commenced in the R and S control (0 mg Cd/L) and cadmium-fed (1 to 10 mg Cd/L) groups to the same extent. Eightypercent of the rats in each group was affected by this pneumonia which was a well-known, well described entity in rats (Dahl and Schackow, 1964), since it was the scourge of most long-term studies with these animals. By week 30,

FIGURE 17

Effect of chronic cadmium feeding on blood pressure in female R and S rats on low salt diet. Each bar represents the mean $\pm$ SEM of 10 animals. Four, 12, 20 and 28 weeks indicate time intervals since the first exposure to cadmium at weaning (3 weeks of age).

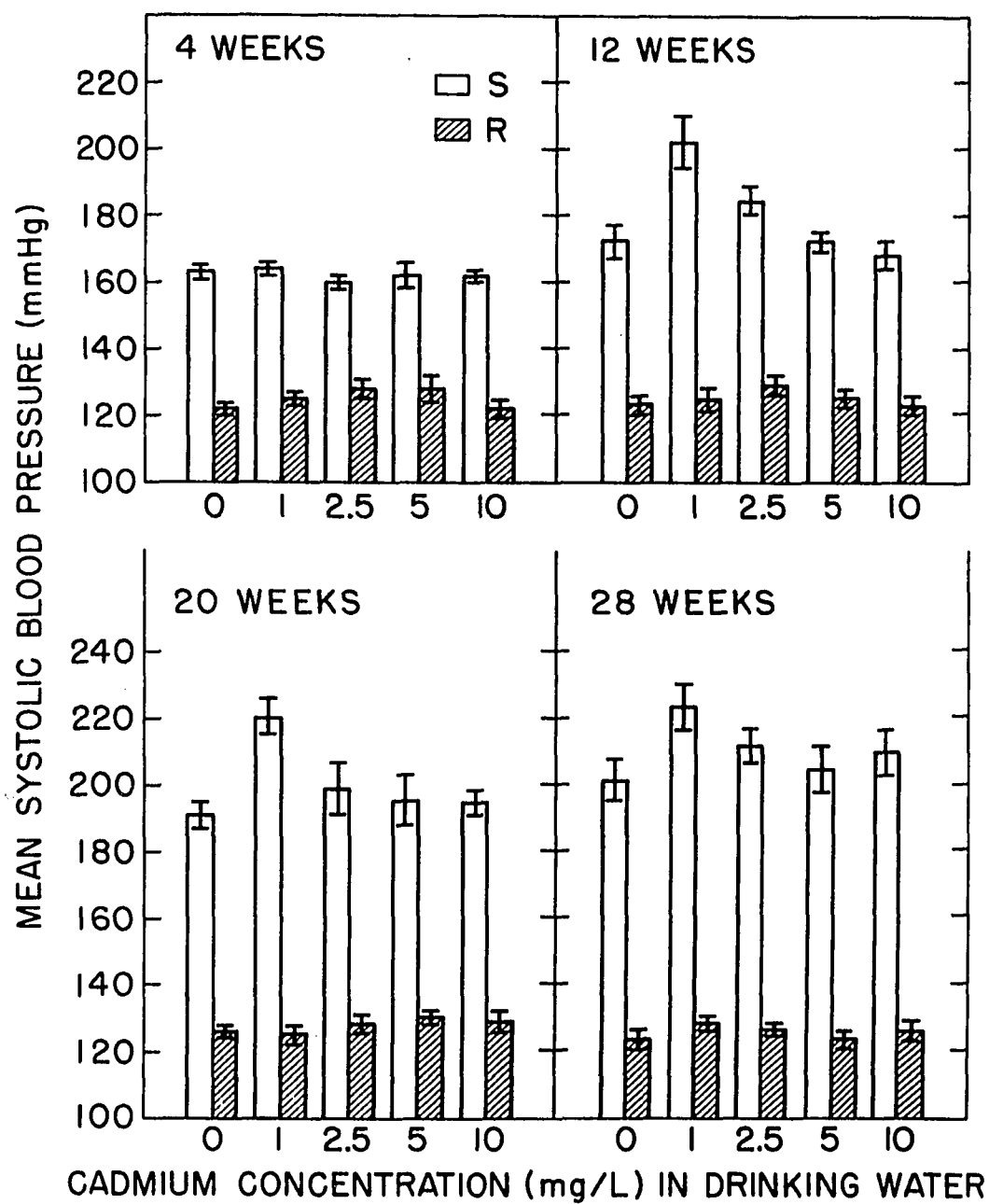


there was no healthy survivors in the five groups to make significant comparison thereafter.

(b) High Salt Diet (Figure 18). Female S rats in the control group, as expected, developed fulminating hypertension whereas the R control (0 mg Cd/L) and cadmium (Cd) groups (1 to 10 mg/L) remained normotensive throughout the study. Among S rats exposed to cadmium (1 to 10 mg/L), blood pressure, as compared with their corresponding control group was not significantly changed ($p > 0.05$) after 4 weeks of cadmium ingestion. S rats fed 1 and 2.5 mg Cd/L manifested significant elevation of blood pressure which differed significantly ($p < 0.01$) from the S control group following 12 weeks of observation. However, after both 20 and 28 weeks of cadmium feeding, only S rats receiving the smallest dose of cadmium (1 mg/L) had significant increase in blood pressure ($p < 0.01$). S rats in the groups exposed to high doses of cadmium (5 and 10 mg/L) behaved differently: the course of their hypertension replicated with remarkable similarity that in the S control group. At week 29, R and S rats began to look sick - decreased motor activity, shivering, poor care of the coat, and ruffled fur - irrespective of cadmium ingestion. These changes were attributed to bronchopneumonia (Dahl and Schackow, 1964) and they were more dramatic in the S strain than in the counterpart R strain. The morbidity of salt-induced hypertension was probably responsible for the difference between the strains. Among the five groups (0 to 10 mg Cd/L), by the 29th week, of the original 20 rats (10 R and 10 S), only 5 (3 R and 2 S), remained healthy. Hence, the experiment was terminated.

FIGURE 18

Effect of chronic cadmium feeding on blood pressure in female R and S rats on high salt diet. Each bar represents the mean $\pm$ SEM of 10 animals. Four, 12, 20 and 28 weeks indicate time intervals since the first exposure to cadmium at weaning (3 weeks of age).



Of those rats examined post mortem, 30% of S animals in each group showed incidence of polyarteritis nodosa. Grossly, the lesions were detected in the mesenteric arteries. They usually formed tortuous, adherent, nodular masses. The degree of involvement was extremely variable and ranged from 3 mm nodule to extensive accumulation of nodules which included the entire mesenteric arterial system. The diameter of the nodules ranged from 1 to 10 mm. No instance of polyarteritis nodosa occurred in R rats in the control and cadmium-fed groups.

3. Effect of Cadmium Feeding on Body and Organ Weights.

(a) Body Weight: It was followed as a simple but sensitive measure of both general well-being and absence of specific toxic effects in the growing female rat. The pertinent body weight data are summarized in Table 11. There was no significant difference ($p > 0.05$) in the body weight among the five groups of either strain on low and high salt diets.

(b) Organ Weights: Within either diet condition, no significant differences ($p > 0.05$) in hepatic or liver weights were noticed between female R and S rats. Hence, these data were not tabulated. In terms of percent of body weight, the kidney and liver weights (200 R and S rats) were: 0.38 ± 0.05 and 4.1 ± 0.6 g respectively, with no cadmium group differing significantly ($p > 0.05$) from the control group. Since no significant difference ($p > 0.05$) appeared between weights (kidney and liver) obtained from animals (R and S) in the

TABLE 11. EFFECT OF CADMIUM FEEDING ON BODY WEIGHT IN FEMALE R AND S RATS

DIET	CADMIUM IN DRINKING WATER (mg/L)	BODY WEIGHT (g)							
		WEEKS (POSTWEANING)							
		4		12		20		28	
LOW SALT	0 ^Δ	163.1	± 5.4 [□]	232.6	± 5.2	256.9	± 5.0	278.3	± 4.3
	1	159.8	± 10.2	237.4	± 5.3	260.6	± 7.3	279.4	± 4.5
	2.5	156.9	± 8.4	234.5	± 6.0	258.3	± 8.0	282.3	± 6.4
	5	149.7	± 7.5	237.7	± 4.1	263.3	± 4.3	280.8	± 4.5
	10	152.6	± 7.3	230.6	± 5.1	253.2	± 4.1	277.5	± 5.3
HIGH SALT	0 ^Δ	158.2	± 6.2	239.4	± 6.0	266.2	± 4.5	284.6	± 2.8
	1	159.9	± 6.4	235.2	± 7.3	264.9	± 5.4	286.2	± 3.0
	2.5	156.8	± 5.1	230.7	± 10.0	260.4	± 3.3	278.8	± 5.0
	5	160.8	± 6.4	236.5	± 6.3	270.3	± 5.3	289.5	± 4.2
	10	154.8	± 6.3	235.4	± 7.2	267.4	± 6.5	278.1	± 7.1
LOW SALT	0 ^Δ	158.5	± 7.1	233.3	± 7.0	251.1	± 5.4	276.9	± 2.5
	1	160.8	± 3.3	232.5	± 4.5	259.6	± 5.2	276.5	± 4.3
	2.5	156.9	± 7.3	229.4	± 5.0	252.4	± 6.2	281.7	± 6.1
	5	151.8	± 10.4	229.4	± 5.2	255.8	± 4.1	275.2	± 5.0
	10	152.1	± 6.1	231.3	± 3.4	249.8	± 7.1	272.5	± 6.2
HIGH SALT	0 ^Δ	159.3	± 4.4	232.4	± 6.4	258.5	± 10.3	279.0	± 5.4
	1	155.2	± 5.2	230.5	± 4.5	258.9	± 3.4	280.8	± 5.5
	2.5	154.9	± 5.4	232.5	± 8.1	264.9	± 7.4	283.1	± 6.3
	5	156.7	± 5.2	231.5	± 5.3	262.8	± 3.2	283.1	± 4.4
	10	155.3	± 8.0	230.8	± 7.1	258.2	± 6.2	286.2	± 5.6

Δ = Control group.

□ = Mean ± SEM of 10 animals.

Low Salt = 0.4% NaCl.

High Salt = 4% NaCl.

control and cadmium-groups, these values were combined. On the other hand, S rats had significantly heavier ($p < 0.001$) adrenals and hearts than R rats irrespective of the diet and cadmium exposure. These data are presented in Table 12. Significant gross cardiac enlargement was only observed in the S rats exposed to the lowest dose of cadmium (1 mg/L) compared to the corresponding S control group ($p < 0.01$).

4. Effect of Cadmium Feeding on Tumor Formation.

Since cadmium has been reported to be both carcinogenic (Gunn et al., 1967) and not associated with an increased incidence of tumors, either benign or malignant (Kanisawa and Schroeder, 1969a), the total incidence of grossly evident tumors in both living and autopsied female R and S rats was recorded. The 40 control rats (R and S on either diet) and the 160 rats with the four cadmium exposures had similar incidences. At 12, 20 and 28 weeks, the cumulative incidences for these 200 rats were 2, 8, and 12%, respectively. There were only large mammary tumors.

5. Effects of Cadmium Feeding on Blood Urea Nitrogen and Plasma Cholesterol.

(a) Blood Urea Nitrogen.

Azotemia, defined as a blood urea nitrogen level of more than 50 mg/100 ml blood, did not occur in any of the control and cadmium groups on either diet as shown in Table 13. Hence, there was no gross impairment of renal function. Furthermore, comparison of the mean blood urea nitrogen values in animals (R and S) fed cadmium and their respective controls were not significantly different ($p > 0.05$).

TABLE 12.

EFFECT OF CADMIUM FEEDING ON ORGAN WEIGHT IN FEMALE R AND S RATS

DIET	CADMIUM IN DRINKING WATER (mg/L)	ORGAN WEIGHT* (mg/100g BODY WEIGHT)			
		ADRENALS		HEART	
		R	S ^Δ	R	S ^Δ
LOW SALT (0.4% NaCl)	0 (CONTROL)	19.5 ± 0.5 [□]	25.3 ± 0.6	283.4 ± 7.2	313.3 ± 6.4
	1	19.1 ± 0.6	24.2 ± 0.5	272.4 ± 10.1	338.6 ± 7.4 [†]
	2.5	20.6 ± 0.5	24.4 ± 0.3	278.3 ± 8.2	314.3 ± 6.2
	5	20.1 ± 0.7	25.1 ± 0.2	286.2 ± 6.4	309.7 ± 4.3
	10	19.2 ± 0.9	23.9 ± 0.7	279.1 ± 10.6	310.5 ± 10.2
HIGH SALT (4% NaCl)	0 (CONTROL)	19.6 ± 0.7	26.0 ± 0.5	286.1 ± 10.7	384.2 ± 6.2
	1	19.4 ± 0.5	25.1 ± 0.7	280.0 ± 6.3	390.3 ± 7.3
	2.5	20.3 ± 0.4	24.9 ± 0.6	279.3 ± 7.3	392.4 ± 10.7
	5	20.6 ± 0.6	24.7 ± 0.9	275.4 ± 11.4	381.4 ± 5.5
	10	20.9 ± 0.8	25.8 ± 0.4	283.3 ± 4.2	379.5 ± 11.5

* The kidney and liver weights were similar in the two strains. In addition, no differences were found between the control and cadmium-fed groups.

Δ Differ from R rats irrespective of the diet and cadmium ingestion ($p \leq 0.001$).

□ Mean ± SEM of 10 animals.

† Differs from the control group ($p < 0.01$).

**TABLE 13. EFFECTS OF CADMIUM FEEDING ON BLOOD UREA NITROGEN AND PLASMA CHOLESTEROL
IN FEMALE R AND S RATS**

DIET	CADMIUM	WEEKS (POSTWEANING)			
	IN	4		28	
	DRINKING	<u>BLOOD UREA NITROGEN</u>			
	WATER (mg/L)	(mg/100 ml)			
		R	S	R	S
LOW SALT	0 ^Δ	26.2 ± 1.4 [□]	30.3 ± 3.5	30.3 ± 3.7	32.8 ± 2.9
	1	28.6 ± 2.9	30.7 ± 3.4	31.9 ± 4.2	34.3 ± 3.4
	2.5	28.0 ± 3.4	28.7 ± 1.9	28.5 ± 3.5	33.2 ± 4.6
	5	30.6 ± 3.1	28.6 ± 2.4	33.0 ± 3.8	34.0 ± 4.0
	10	29.1 ± 4.3	31.2 ± 2.3	31.0 ± 2.9	32.1 ± 3.7
HIGH SALT	0 ^Δ	29.2 ± 2.2	32.3 ± 2.4	30.1 ± 3.2	30.0 ± 5.5
	1	30.7 ± 2.6	30.0 ± 3.1	29.9 ± 3.6	30.2 ± 3.2
	2.5	31.9 ± 3.4	30.8 ± 1.7	29.0 ± 4.1	31.2 ± 4.9
	5	30.1 ± 2.1	31.2 ± 2.3	28.3 ± 3.8	33.1 ± 6.6
	10	31.7 ± 2.8	33.0 ± 2.0	31.4 ± 2.8	29.1 ± 3.4
<u>CHOLESTEROL (mg/100 ml)</u>					
LOW SALT	0 ^Δ	88.8 ± 3.6	94.0 ± 4.3	92.8 ± 6.5 [†]	96.2 ± 5.5 [†]
	1	92.0 ± 3.7	95.7 ± 4.0	74.6 ± 5.6	80.1 ± 4.7
	2.5	89.9 ± 4.0	94.8 ± 3.6	83.4 ± 3.9	83.4 ± 5.1
	5	89.8 ± 5.0	97.0 ± 6.3	80.1 ± 5.7	81.4 ± 3.2
	10	86.2 ± 6.0	96.1 ± 4.1	82.7 ± 5.8	78.2 ± 4.1
HIGH SALT	0 ^Δ	87.7 ± 3.5	98.2 ± 6.2	82.4 ± 5.1	124.0 ± 10.3 [*]
	1	89.6 ± 4.7	105.0 ± 4.3	85.0 ± 3.2	131.1 ± 7.2 [*]
	2.5	97.2 ± 3.0	100.8 ± 7.9	76.8 ± 6.7	125.6 ± 9.3 [*]
	5	85.3 ± 4.3	96.7 ± 5.0	85.2 ± 5.3	134.2 ± 10.3 [*]
	10	92.2 ± 5.0	94.7 ± 7.5	84.6 ± 5.7	119.5 ± 10.7 [*]

Δ = Control group.

□ = Mean ± SEM of 10 animals.

† = Differs from cadmium groups ($p < 0.01$).

* = Differs from the corresponding R group ($p < 0.01$).

Low Salt = 0.4% NaCl

High Salt = 4% NaCl

(b) Plasma Cholesterol.

On week 4, statistical analysis showed that the differences of plasma cholesterol values were without significance ($p > 0.05$) for all groups on either diet (Table 13). By contrast, on week 28 the following changes occurred: (1) Low salt diet: R and S rats given cadmium (1 to 10 mg/L) exhibited significantly depressed ($p < 0.01$) levels of plasma cholesterol compared with R and S controls; and (2) High salt diet: S rats in the control and cadmium groups (1 to 10 mg/L) had significant elevation ($p < 0.01$) of plasma cholesterol levels compared with the corresponding R rats in the control and cadmium groups.

6. Effect of Cadmium Feeding on Urinary pH, Glucose, Blood and Protein.

Cadmium did not affect pH of the urine significantly ($p > 0.05$), the mean being 5.80. Glycosuria and hematuria were not detected in any of the R and S control and cadmium-fed groups on either diet. Therefore, these data were not discussed.

The pertinent urinary protein data are summarized in Table 14. Proteinuria was significantly more marked ($p < 0.01$) in S rats than their counterpart R rats irrespective of the diet and cadmium exposure. After 28 weeks of observation, S cadmium groups (1 to 10 mg/L) on low salt diet developed significant proteinuria ($p < 0.01$) compared with the corresponding S controls. However, the difference of the means were without significance ($p > 0.05$) for (a) S rats in the control and cadmium-fed groups on low salt diet on week 4; (b) S rats in the control and cadmium-fed groups on high salt diet on weeks 4 and 28; and (c) R rats in the control and cadmium-fed groups on either diet on weeks 4 and 28.

TABLE 14.

EFFECT OF CADMIUM FEEDING ON URINARY PROTEIN CONCENTRATION IN FEMALE R AND S RATS*

DIET	CADMIUM IN DRINKING WATER (mg/L)	URINARY PROTEIN (mg/100 ml)			
		4 WEEKS (POSTWEANING)		28	
		R	S ^Δ	R	S ^Δ
LOW SALT (0.4% NaCl)	0 (CONTROL)	55 ± 15 [□]	140 ± 20	65 ± 11	160 ± 30
	1	46 ± 15	140 ± 26	65 ± 11	260 ± 26 [†]
	2.5	46 ± 15	140 ± 26	65 ± 11	260 ± 26 [†]
	5	64 ± 14	140 ± 26	55 ± 15	260 ± 26 [†]
	10	64 ± 14	140 ± 26	65 ± 11	260 ± 26 [†]
HIGH SALT (4% NaCl)	0 (CONTROL)	65 ± 11	180 ± 32	65 ± 11	650 ± 116
	1	58 ± 11	200 ± 33	55 ± 15	650 ± 116
	2.5	51 ± 10	200 ± 33	65 ± 11	650 ± 116
	5	65 ± 12	180 ± 32	65 ± 11	650 ± 116
	10	58 ± 11	180 ± 32	65 ± 11	650 ± 116

* Hematuria and glycosuria were not detected in the female R and S control and cadmium groups.

Δ Differ from R rats irrespective of the diet and cadmium exposure ($p < 0.01$).

□ Mean ± SEM of 10 animals.

† Differs from the control group ($p < 0.01$).

7. Cadmium Concentrations in Tissues.

The observed renal and hepatic cadmium concentrations are presented in Table 15. Since no significant differences appeared between left and right renal tissues, these values were pooled. Doubling the intensity of the exposure very roughly doubled the renal and hepatic cadmium concentrations. The levels of cadmium in the kidney and liver tissues in S rats fed cadmium (1 to 10 mg/L) were significantly higher ($p < 0.001$) than that of R rats on either diet.

Combining the data on systolic pressure levels and renal cadmium concentrations of R and S rats on low salt diet suggested that, under the experimental conditions used here, concentrations of 10 to 38 μg of cadmium per gram of S kidney were generally associated with elevated pressures; whereas higher and lower concentrations generally were not.

8. Renal Vascular and Hepatic Changes in Response to Cadmium Feeding.

(a) Kidney: Renal vascular changes were hitherto classed as "nephrosclerosis". Slight changes consisted of hyalinization of the glomerular tuft with a well-defined thickening of the basement membrane surrounding the glomerulus, a discernible degree of tubular dilatation, and increased thickening of the media of small arterioles. These changes, which for convenience were classified as one-plus (+), were usually patchy in distribution. Moderate, or two-plus (++), changes were those where glomerular hyalinization was progressed to the point of obliterating the glomerulus; tubular dilatation was prominent and casts were numerous. These changes, together with the vascular change, while still patchy were widely distributed throughout the

TABLE 15. CADMIUM CONCENTRATIONS IN THE KIDNEY AND LIVER OF FEMALE R AND S RATS
FED CADMIUM FOR 28 WEEKS

DIET	CADMIUM IN DRINKING WATER (mg/L)	CADMIUM CONCENTRATION ($\mu\text{g/g}$ WET WEIGHT)					
		KIDNEY*				LIVER	
		R		S [†]		R	S [†]
LOW SALT (0.4% NaCl)	0 (CONTROL)	0.76	$\pm$ 0.03 [□]	0.78	$\pm$ 0.05	0.28 $\pm$ 0.02	0.31 $\pm$ 0.03
	1	5.71	$\pm$ 1.53	10.6	$\pm$ 4.93	3.43 $\pm$ 1.31	7.58 $\pm$ 1.41
	2.5	10.2	$\pm$ 3.76	20.9	$\pm$ 4.62	4.51 $\pm$ 1.20	9.32 $\pm$ 2.14
	5	16.4	$\pm$ 2.31	38.4	$\pm$ 10.1	11.3 $\pm$ 3.06	23.4 $\pm$ 6.40
	10	22.3	$\pm$ 3.47	82.7	$\pm$ 14.3	17.4 $\pm$ 8.10	68.1 $\pm$ 5.13
HIGH SALT (4% NaCl)	0 (CONTROL)	0.90	$\pm$ 0.14	0.87	$\pm$ 0.18	0.37 $\pm$ 0.10	0.41 $\pm$ 0.21
	1	6.85	$\pm$ 1.18	14.8	$\pm$ 2.13	4.27 $\pm$ 0.12	10.6 $\pm$ 2.32
	2.5	13.3	$\pm$ 3.72	27.2	$\pm$ 4.31	6.76 $\pm$ 1.13	14.9 $\pm$ 2.11
	5	20.6	$\pm$ 5.18	55.7	$\pm$ 9.10	14.6 $\pm$ 4.71	30.6 $\pm$ 8.84
	10	30.2	$\pm$ 3.02	102.7	$\pm$ 15.3	22.3 $\pm$ 4.10	70.2 $\pm$ 12.3

* = Left and right kidneys were pooled.

† = Differ from the corresponding R cadmium rats ($p < 0.001$).

□ = Mean $\pm$ SEM of 10 animals.

parenchyma. Marked, or three-plus (+++) changes, while the same in character as the foregoing, were generalized in distribution so that only rarely was normal kidney structure encountered. A summary of the renal vascular changes in female R and S rats is shown in Table 16. S rats on low salt diet and exposed to cadmium (1 to 5 mg/L) exhibited significantly more changes ($p < 0.01$) than the respective S controls and S rats fed the highest dose of cadmium (10 mg/L). By contrast, renal vascular changes occurred in S rats in the control and cadmium groups (1 to 10 mg/L) on high salt diet to the same extent. No significant changes were observed in R strain groups on either diet.

The pathological changes in the kidney appeared to be associated with either cadmium or salt-induced hypertension.

(b) Liver: Hepatic morphological changes (cirrhosis, fatty degeneration and vacuolation of hepatic cells) were graded as follows: normal = 0, slight = +, moderate = ++ and marked = +++. Since mild to moderate hepatic changes were observed in R and S rats in the control and cadmium-fed groups on either diet, these data are not presented.

9. Effect of Cadmium Feeding on Plasma Renin Activity.

The plasma renin activity data are shown in Table 17. The effect of strain was significant, with female R rats having much higher plasma renin activity than female S rats ($p < 0.001$). The effect of diet was also significant, with rats fed low salt having higher plasma renin activity than rats fed high salt ($p < 0.001$).

TABLE 16.

RENAL VASCULAR CHANGES IN FEMALE R AND S RATS FED CADMIUM FOR 28 WEEKS*

CADMIUM IN DRINKING WATER (mg/L)	STRAIN (N)	LOW SALT DIET (0.4% Na Cl) NEPHROSCLEROSIS			HIGH SALT DIET (4% NaCl) NEPHROSCLEROSIS		
		0	+-+	+++	0	+-+	+++
0 (Control)	R (10)	10	0	0	9	1	0
	S (10)	7	3	0	0	3	7 ^Δ
1	R (10)	9	1	0	10	0	0
	S (10)	3	7 [□]	0	0	2	8 ^Δ
2.5	R (10)	9	1	0	8	2	0
	S (10)	1	8 [□]	1	0	2	8 ^Δ
5	R (10)	8	2	0	9	1	0
	S (10)	1	7 [□]	2	0	2	8 ^Δ
10	R (10)	9	1	0	9	1	0
	S (10)	7	3	0	0	1	9 ^Δ

* Since mild to moderate hepatic changes were observed in both control and cadmium groups, these data are not tabulated.

N = Number of rats examined, 0 = normal, +-+ = slight to moderate changes, +++ = marked changes.

Δ = p < 0.01 compared with R strain rats.

□ = p < 0.01 compared with R strain rats and S strain controls.

TABLE 17.

PLASMA RENIN ACTIVITY IN FEMALE R AND S RATS FED CADMIUM
FOR 28 WEEKS

CADMIUM IN DRINKING WATER (mg/L)	PLASMA RENIN ACTIVITY (ng/ml/hr)			
	LOW SALT DIET (0.4 % NaCl)		HIGH SALT DIET (4% NaCl)	
	R ^Δ	S	R ^Δ	S
0 (Control)	24.1 ± 4.22 [□]	10.3 ± 2.61	20.2 ± 3.61	5.21 ± 1.06
1	22.5 ± 5.10	12.4 ± 3.01	19.0 ± 4.32	4.74 ± 1.12

Δ Differ from S rats ($p < 0.001$).

□ Mean ± SEM of 10 animals.

These results confirm earlier observations by Iwai et al. (1973) who showed that S rats had lower plasma and kidney renin activities than did R rats. In this study cadmium ingestion per se did not alter plasma renin activity levels in both strains on either diet.

V. DISCUSSION

Cadmium (Cd) has been implicated as a possible factor in hypertension and arteriosclerotic heart disease by Schroeder and other investigators (Carroll, 1966; Perry and Schroeder, 1955; Schroeder, 1964b; Schroeder, 1965; Schroeder, 1967; Schroeder, 1969; Schroeder and Balassa, 1961; Schroeder et al., 1967). Tipton and co-workers (Tipton and Cook, 1963; Tipton et al., 1965) did not detect cadmium in the kidney and other tissues of newborn infants, whereas it was invariably present in the corresponding adult tissues. Cadmium is ingested by man in varying amounts (200-400 $\mu\text{g/day}$) in the food, drinking water, and environmental air (Carroll, 1966; Schroeder, 1965; Schroeder, 1967; Schroeder and Balassa, 1961; Schroeder et al., 1967). Hardness of the municipal water (Anderson et al., 1969; Schroeder, 1960; Schroeder, 1969) was inversely correlated with, and cadmium concentration in the air (Carroll, 1966) was directly correlated with, the death rates from hypertension and arteriosclerotic heart disease. Experimental hypertension has been consistently produced by feeding small amounts of cadmium in the drinking water (Perry and Erlanger, 1973; 1974; Schroeder, 1964a; Schroeder and Balassa, 1961; Schroeder et al., 1968; Schroeder and Vinton, 1962; Schroeder et al., 1963) or by parenteral cadmium administration (Schroeder and Buckman, 1967; Schroeder et al., 1968) in the rat. However, some investigators did not observe a relationship between cadmium and hypertension (Doyle et al., 1975; Morgan, 1969; Lener and Bibr, 1970; 1971; Lewis et al., 1972; Porter, 1974; Tsuchiya,

1969). Therefore, it appeared to be desirable to design acute and chronic cadmium experiments to explore the genetic influence on the effects of cadmium on blood pressure and other parameters in Dahl hypertension resistant (R) and Dahl hypertension-sensitive (S) strains of rats.

Perry and co-workers (Perry et al., 1967; Perry and Yunice, 1965) produced acute diastolic hypertension in anesthetized female Sprague-Dawley rats by intra-arterial injection of cadmium (0.1 and 0.4 mg/kg). However, larger doses (0.8-3.20 mg/kg) of cadmium were depressors. To ascertain if reactivity of blood vessels in male Long-Evans rats made hypertensive by feeding or injecting cadmium was altered, the vascular responses to intravenously injected angiotensin II and norepinephrine were studied by Schroeder et al. (1970). Rats, fed 10 mg Cd/L in water for two to four months, had depressed response to angiotensin II and norepinephrine. In addition, cadmium, intraperitoneally injected in normal rats, rendered vascular reactivity less sensitive to angiotensin II and norepinephrine. Dahl et al. (1964) showed that among normotensive R and S rats, all maintained on the same low salt diet, S rats had significantly greater reactivity to both angiotensin II and norepinephrine than did R rats, and salt ingestion per se was not found to influence vascular reactivity in these rats.

The purpose of our Acute Cadmium Study was to determine if cadmium, another vasoconstrictor substance, differentially affected R and S rats and the following observations were made: (a) intra-

arterial injections of cadmium (0.1 and 0.4 mg/kg) produced an immediate decrease in blood pressure to below the baseline followed by a rapid increase to a level above the baseline; (b) the acute pressor response to intra-arterially injected cadmium (0.4 mg/kg) in the S rat was twice as high as in the R rat; and (c) cadmium (0.4 mg) did not sensitize the blood vessels to the action of angiotensin II (20 and 40 ng/kg) or norepinephrine (200 and 400 ng/kg) in R and S rats. On the contrary, the presence of cadmium in R rats only significantly depressed vascular responses to angiotensin II and norepinephrine.

It is noteworthy that Perry and co-workers (Perry et al., 1967; Perry and Yunice, 1965) failed to detect the immediate decrease in blood pressure (which lasted approximately 25 seconds in our study) while injecting low doses of cadmium (0.1 and 0.4 mg/kg, intra-arterially) because in their experiment the total time of cadmium injection and subsequent flushing with 0.9% aqueous NaCl was 30 seconds, during which blood pressure was not recorded. It is also worth emphasizing that blood pressure was monitored continuously in our Acute Cadmium Study. At this juncture, there are no possible explanations for the differences in acute blood pressure responses to pre and post-cadmium angiotensin II or norepinephrine injections between R and S rats. In summary, this study demonstrated that the vascular response to cadmium was, as with other hypertensinogenic stimuli, determined to a great extent by genetic predisposition.

Schroeder et al. (1966) showed that intraperitoneal injection of cadmium (1 and 2 mg/kg) resulted in significant arterial hypertension in adult female Long-Evans rats to a degree equalling or

exceeding that resulting from constriction of one renal artery. The duration of the hypertension did not exceed one month. However, we observed (Chronic Cadmium Study 1) that when weanling R and S rats of both sexes were given cadmium by intraperitoneal injections: (a) significant and persistent (16 weeks) hypertension developed in female S rats but not in R rats of either sex. Although cadmium failed to produce consistent hypertension in male S rats, it did increase their mortality rate; (b) the concentrations of cadmium in the hepatic and renal tissues of cadmium-injected S rats of both sexes were significantly higher than those of R rats; and (c) the female S kidney exposed to cadmium by injection showed renal vascular changes well in accord with renal hypertension of moderate degree.

Schroeder and Vinton (1962) observed the development of hypertension in young female rats given small amounts of cadmium in drinking water from the time of weaning. Conversely, male rats were largely resistant to this disorder, which appeared later in life. They had no explanation for this difference. Similarly, female S rats injected with cadmium manifested an increase in blood pressure, whereas the R rats and the male S rats showed no changes in blood pressure. In contrast to these results, previous studies in this laboratory showed that the rate and the degree of pressure-increase as well as the malignancy of disease afflicting S animals depended on the amount of salt added and the age at which it was started. The heavier the exposure and the earlier in life it began, the more malignant the disease (Knudsen and Dahl, 1966). While the ultimate blood pressure levels were similar in both sexes on a high sodium chloride diet, the

males generally developed hypertension more rapidly than the females (Dahl et al., 1975), and castration of males had no effect on blood pressure (Dahl et al., 1975). At the present time, insufficient data are available to explain the observed sex difference in the blood pressure response to cadmium in the rats.

There has been great interest in the structure and function of metallothionein (Kägi and Vallee, 1960), a cadmium-binding protein which may play an important role in cadmium detoxication. This protein contains about 30% cysteinyl residues and consequently has an extremely high metal-binding potential, with one metal atom bound/3-SH groups. It may contain up to 9% metal with a log stability constant of the 3:1 cadmium-thionein complex of 25.5. Zinc is usually a major, and copper and mercury minor components of the protein from equine and human renal cortex (Kägi and Vallee, 1960; Pulido et al., 1966). Metallothionein, which is generally heterogeneous, was also isolated and characterized from the livers of horse, (Kägi et al., 1974) rabbit (Nordberg et al., 1972) and rat (Winge and Rajagopalan, 1972). There were reports of the occurrence in rats and mice of a cadmium-binding protein of similar molecular weight (around 10,000) in testes (Chen et al., 1972; Nordberg, 1971), blood (Nordberg et al., 1971) and urine (Nordberg and Piscator, 1972). It is possible, but not yet proved, that this protein is similar to metallothionein. The limited evidence available suggests that the metal-free thionein is absent from rat liver and kidney and that the appearance of cadmium bound to this protein after cadmium injection results from its de novo synthesis (Shaikh and Lucis, 1970; 1972;

Webb, 1972a; 1972b).

The reason(s) for the striking and unexpected difference in cadmium-binding between R and S rats might be (a) a difference in the concentration of metallothionein and/or (b) a difference in cadmium-binding properties of metallothionein.

Gunn and Gould (1957) gave a single intracardiac injection of ^{115}Cd -nitrate to rats and followed the animals for up to 8 months. Their data showed that during the first month there was a decrease in liver levels of ^{115}Cd and an increase in kidney levels, so that after a month of exposure, the concentration in the renal cortex was the same as that in the liver. Renal medulla, had considerably lower ^{115}Cd uptake than renal cortex. The same distribution pattern was observed in S and R rats (Acute Cadmium Study 2). However, in S rats exposed to cadmium (1 and 2 mg/kg) injection, the selective accumulation of cadmium in the renal cortex and medulla and liver was significantly higher than that of R rats at the onset of cadmium-induced hypertension.

Schroeder et al. (1966) failed to detect renal vascular changes in the kidneys of cadmium-injected hypertensive rats. By contrast, female S rats showed mild to moderate renal vascular changes in response to cadmium-induced hypertension. Furthermore, the pathological changes in the S kidney were not necessarily correlated with renal cadmium content (which was similar in both male and female S rats) but they appeared to be the result of cadmium-induced hypertension.

In Chronic Cadmium Study 3, adult, instead of weanling, male and female R and S rats were exposed to cadmium (2 mg/kg) in order to ascertain if the rate and the degree of blood pressure-increase in response to cadmium were age-dependent in S rats. Contrary to the findings in male weanlings (Chronic Cadmium Study 1) it was noted that cadmium produced sustained hypertension in adult male as well as female S rats. The cadmium-induced elevation of blood pressure in female S rats was more pronounced than in male S rats and the renal vascular and hepatic changes induced by cadmium injection --- not present in hypertensive Long-Evans rats (Schroeder et al., 1966) --- were detectable in S rats of either sex to the same extent. Hence, cadmium-induced hypertension associated with renal vascular changes was age-dependent in S males.

Dahl and Heine (1960) reported that the hypertension produced in Sprague-Dawley females by feeding sea salt was more pronounced than that caused by sodium chloride alone, although the average amount of sodium chloride contained in the sea salt feeding was slightly less. They suspected that other ions may add to the severity of hypertension induced by excess dietary sodium. However, they did not indicate which ion or ions may be involved in the enhancement of the hypertensive process in rats. The enhancement of the hypertensinogenic effect of sodium chloride induced by cadmium (1 and 2 mg/kg) injection (Acute Cadmium Study 4) suggests the possibility that the presence of cadmium in sea salt might have been the factor which potentiated hypertension in Sprague-Dawley rats.

Schroeder and his co-workers (1966) reported that intraperitoneal cadmium injection (2 mg/kg) in conjunction with renal artery constriction produced arterial hypertension in female Long-Evans rats to a degree equalling that resulting from renal artery constriction alone. In our investigation of the influence of cadmium (2 mg/kg) injection on two-kidney Goldblatt hypertension in male R and S rats (Chronic Cadmium Study 5), we found that S rats injected with cadmium developed more severe hypertension than the saline-injected controls. Thus, the morbidity and mortality of two-kidney Goldblatt hypertension in the S rat were exaggerated by cadmium administration. The reason for the discrepancy between Schroeder's study (Schroeder et al., 1966) and the present study is not clear at this time.

Schroeder suggested that at least part of human "essential" hypertension results from inapparent but universal exposure to cadmium. Support for this hypothesis was found in his observation that hypertension developed in rats which were chronically fed small amounts of cadmium from weaning until death (Schroeder, 1964; Schroeder and Balassa, 1961; Schroeder et al., 1968; Schroeder and Vinton, 1962; Schroeder et al., 1963). In summary, Schroeder and his colleagues found that: (a) Long-Evans rats receiving small amounts of cadmium in drinking water (5 mg Cd/L) exhibited systolic hypertension, the incidence being greater in females than in males. In males the disorder appeared later in life; (b) the hypertension was usually associated with increased mortality and renal vascular changes; and (c) signs of chronic cadmium toxicity were not observed at this dose

of cadmium (5 mg/L). Incisor teeth were not bleached, anemia was not evident, growth rates were similar to the controls. Systolic hypertension was the only overt "toxic" manifestation of cadmium ingestion in the female animals. In a later study, however, Kanisawa and Schroeder (1969b) reported that hypertensive cadmium-fed (5 mg/L) Long-Evans rats exhibited renal vascular lesions, cardiac hypertrophy and proteinuria. Using conditions very similar to Schroeder's, Perry and Erlanger (1974) found that 1, 2.5 and 5 mg cadmium per liter of drinking water reproducibly induced an elevation of systolic pressure whereas rats given 10 and 25 mg Cd/L had smaller increases in pressure, and rats given 50 mg Cd/L were sick and had significant decreases in pressure. Furthermore, cadmium (5 mg/L) in drinking water was associated with increased plasma renin activity which was significant after 1 week and 1 month but not after three months of exposure to cadmium (Perry and Erlanger, 1973). The results of our Chronic Cadmium Study 6 confirmed and extended only the following observations of Schroeder and Perry. (1) Cadmium (1 to 5 mg/L) in the drinking water proved to be hypertensive in female S rats on low salt diet. Blood pressure elevation seemed to be highest in S rats receiving the lowest dose of cadmium (1 mg/L) whereas the largest dose (10 mg Cd/L) had no significant effect on pressure. R rats remained normotensive irrespective of the diet and cadmium exposure throughout the study. (2) Cadmium (1 mg/L) accelerated the rate of salt-induced hypertension in S rats. (3) Cadmium suppressed plasma cholesterol levels in either strain on low salt diet. (4) Cadmium had no influence on life span, body weight, tumor formation, susceptibility to infection and plasma renin activity

in either strain on both diets. (5) Cadmium (1 mg/L) caused gross cardiac hypertrophy in S rats on low salt diet. (6) The concentrations of cadmium in the kidney and liver tissues of cadmium-fed S rats on either diet were significantly higher ($p < 0.001$) than that of R rats. (7) Cadmium (1 to 5 mg/L) caused mild to moderate nephrosclerosis in S rats on low salt diet; and (8) Cadmium (1 to 10 mg/L) induced proteinuria in cadmium-fed S rats on low salt diet. The pathogenesis of salt-induced hypertension in S rats on high salt diet apparently obscured the effects of chronic cadmium ingestion observed in S rats on low salt diet.

Cadmium-induced hypertension observed in cadmium-fed (1 to 5 mg/L) S rats on low salt resembles human "essential" hypertension in that it is usually mild or moderate and not accompanied by severe cadmium toxicity; however, even the lowest effective animal exposure to date (Perry and Erlanger, 1974) is about two orders of magnitude above the ordinary human exposure by ingestion which approximates 1 μg per kg of body weight. Despite this disparity, tissue cadmium concentration in exposed S rats are comparable to those in human beings without unusual exposure. Thus, renal cadmium concentration in exposed hypertensive S rats ranges from 10 to 38 μg per g, while the average American adult has about 30 μg per g. The absence of a hypertensive effect in S rats following a high exposure to cadmium (10 mg/L) is consistent with the absence of hypertension in industrial workers absorbing large quantities of cadmium from industrial dusts into the lungs (Schroeder, 1967) and in victims of "Itai-Itai Byo" disease from areas of Japan with a high exposure to cadmium (Tsuchiya,

1969). The observations in S rats also suggest that there may be a critical amount of cadmium which has to accumulate in the kidney in order to induce hypertension, with either too little or too much being ineffective. It is also worth emphasizing that mild hypertension, with blood pressure elevations of only 10 to 20 mmHg is not innocuous but, rather, is associated with a very significant increase in cardiovascular death rates, particularly from myocardial infarction.

The data obtained in S rats fed 1 mg Cd/L and high salt diet may also have important clinical implications since hypertension is multifactorial in origin, that is, there are probably multiple factors that may operate singly or in combination to induce hypertension in an individual with appropriate predisposition. A combination, for instance, of both cadmium, and salt, another environmental contaminant in food, might prove to be a particularly vicious combination in inducing hypertension in genetically predisposed humans.

No mechanism for cadmium-induced hypertension has been determined. Several possible mechanisms can be postulated. Hypertension induced by intra-arterial cadmium was associated with increased cardiac output, at least during the first few minutes after injection in female Sprague-Dawley rats (Perry et al., 1967). The magnitude of cardiac output increase was directly proportional to the magnitude of the cadmium-induced hypertension. Changes in peripheral resistance induced by cadmium were less consistent and more difficult to interpret. Scott and Haddy (1963) and Perry et al. (1967) found cadmium to be vasoactive in the dog and rat, respectively. Total peripheral resist-

ance decreased in the dog forelimb vascular bed (Scott and Haddy, 1963), but an increased arterial resistance was found in the isolated rat hindquarters preparations (Perry et al., 1967). Isolated organ preparation are generally preferred for the study of drug effects, since the influence of drug transference, transport, chemical transformation, excretion, etc., is reduced to a minimum (Ariens et al., 1964). Kleinfeld and Stein (1968) produced a reversible decrease of 50-90% in the contractile tension of the rat atrium preparations with cadmium. Perry and co-workers (1967) found that cadmium irreversibly inhibited the response of rabbit aortic strips to L-norepinephrine but not to angiotensin II. Vander (1962) found that direct left renal artery infusion of cadmium (0.025 mg/kg) produced increased sodium reabsorption in the dog, and he concluded that cadmium had the ability to enhance proximal tubular sodium reabsorption in the dog. Thereafter it was observed (Perry et al., 1971) that normal rats injected with 1 mg cadmium per kilogram of body weight intramuscularly averaged 42 μ Eq less urinary sodium than control animals. This antinatriuretic effect increased with successive doses of 1 mg cadmium per kilogram of body weight intramuscularly, reaching an average of 68 μ Eq after the fourth such dose. Foulke et al. (1974) reported a decrease in sodium excretion in rats given a single intraperitoneal injection of cadmium (10 mg/kg). On the other hand, in similar experiments, Doyle and associates (1975) found sodium retention without hypertension in female Long-Evans rats fed 5 mg Cd/L drinking water.

Schroeder and Balassa (1965) observed that lowered circulating cholesterol was associated with increased aortic lipids in Long-Evans rats given cadmium in drinking water (5 mg/L). Delisle and co-workers (1971) reported increased cardiac norepinephrine in rats fed cadmium (100 mg/L drinking water) for 2 to 10 weeks and ascribed this cardiac effect of cadmium to: (a) a decreased rate of norepinephrine degradation; (b) an increased synthesis of norepinephrine with increased storage; (c) a greater uptake of circulating norepinephrine; or to the combination of any of these factors. Circulating renin increased following both intraperitoneal injection of cadmium (1.8 μ moles) and cadmium feeding (5 mg/L drinking water) in rats (Perry and Erlanger, 1973). However, cadmium-induced elevation of renin activity disappeared in rats after 3 months despite continued cadmium feeding. Daily intraperitoneal administration of cadmium (1 mg/kg) to rats for 45 days significantly increased adrenal weights and augmented the levels of adrenal norepinephrine and epinephrine as well as the activity of adrenal tyrosine hydroxylase (Rastogi and Singhal, 1975). Finally, Perry and Erlanger (1975) suggested that the increase in blood pressure induced by intraperitoneal injection of cadmium (2 μ moles/kg) resulted from a direct effect on vascular smooth muscle. Their suggestion was based on the following observations: (a) with the exception of the "alpha blocker" phentolamine, autonomic drugs (propranolol, practolol and pentolinium) had no significant effect on cadmium-induced hypertension; (b) ethylenediaminetetraacetate had a slight early effect when given after cadmium, but the specific cadmium chelating agent (cyclohexane - 1,2-diamine - NNN'N' -

tetraacetate) was inert; (c) both of the directly acting vasodilators, hydralazine and minoxidil, blocked the cadmium-induced hypertension, and both were more effective when given before cadmium. Thus, these agents may block the receptors associated with the cadmium induction of hypertension; (d) the inhibition of cadmium-induced hypertension by zinc seemed likely to have been competitive; it required a considerable excess of zinc (5-fold), which shortened but did not block the rise in blood pressure. Conversely, selenium completely prevented the hypertension induced by cadmium. The mechanism was uncertain, although competitive inhibition seemed likely; (e) complete constriction of renal blood vessels did not inhibit cadmium-induced hypertension, although it did block renin release, indicating that renin was not involved in the cadmium effect; and (f) established cadmium-induced hypertension was not entirely irreversible, however, since minoxidil induced significant lowering of the blood pressure. The parabiotic studies involving R and S strains (Dahl et al., 1967; Iwai et al., 1969; Knudsen et al., 1969) suggested indirectly that a humoral factor from the S kidney (or requiring the presence of the S kidney) can elevate blood pressure in a parabiont R partner. Later studies with renal homografts (Dahl et al., 1972; 1974) led more directly to the conclusion that in normotensive rats from these strains the blood pressure could be elevated to hypertensive levels or maintained at normotensive levels, depending on whether an S or an R genotype renal homograft was the sole source of renal function. Dahl and Heine (1975) demonstrated that elevated blood pressure could be reduced to normal

levels by a normotensive R kidney in a hypertensive S rat and that a hypertensive S homograft could raise the blood pressure of a normotensive R host. Thus, R and S kidneys play an important, genetically-determined role in the development of hypertension, or lack thereof, following exposure to any one of several hypertensinogenic stimuli commonly used to invoke experimental hypertension. Therefore, it would seem reasonable to speculate that: (a) genetically controlled factors acting through the kidney can chronically modify the blood pressure up or down; and (b) cadmium may act on vascular smooth muscle through the kidneys in order to induce hypertension in S rats.

To conclude, the data cited in the acute and chronic cadmium studies using Dahl hypertension-resistant (R) and Dahl hypertension-sensitive (S) strains of rats, indicated that the genetic substratum was a critical determinant of whether experimental hypertension develops after exposure to cadmium. If these experimental models have bearing on the etiology of "essential" hypertension in man, these studies suggest that even if current environmental cadmium pollution is not of immediate concern to "all" individuals, it may be a genuine health hazard to "sensitive" individuals with a family history of hypertension.

- Ambard, L., and E. Beaujard. 1904. Causes de l'hypertension arterielle. Arch. Gén. Med. 1: 520-522.
- Anderson, T. W., W. H. LeRiche, and J. S. MacKay. 1969. Sudden death and ischemic heart disease. New England J. Med. 280: 805-807.
- Ariens, E. J., A. M. Simonis, and J. M. van Rossum. 1964. Drug-receptor interaction: Interaction of one or more drugs with one receptor system. P. 119-286. In E. J. Ariens (Ed.), Molecular Pharmacology. Academic Press, New York.
- Boucher, R., J. Menard, and J. Genest. 1967. A micromethod for measurement of renin in plasma and kidney of rats. Canad. J. Physiol. Pharmacol. 45: 881-890.
- Carroll, R. E. 1966. The relationship of cadmium in the air to cardiovascular disease death rates. J. Amer. Med. Assoc. 198: 177-179.
- Chen, R., P. Wagner, H. E. Ganther, and W. G. Hoekstra. 1972. A low molecular weight cadmium-binding protein in testes in rats: possible role in cadmium-induced testicular damage. Feder. Proc. 31: 2725 (Abstract).
- Cotzias, G. C. 1967. Importance of trace substances in environmental health as exemplified by manganese. P. 5-19. In D. D.

- Hemphill (Ed.), Trace Substances in Environmental Health-I.
Columbia, Missouri.
- Dahl, L. K. 1958. Salt intake and salt need. New England J.
Med. 258: 1152-1157, 1205-1208.
- Dahl, L. K. 1960. Salt, fat and hypertension: The Japanese
experience. Nutrition Rev. 18: 97-99.
- Dahl, L. K., and M. Heine. 1960. Effects of chronic excess
salt feeding: Enhanced hypertensogenic effect of sea salt
over sodium chloride. J. Exptl. Med. 113: 1067-1076.
- Dahl, L. K., and M. Heine. 1975. Primary role of renal homografts
in setting chronic blood pressure levels in rats. Circul. Res.
36: 692-696.
- Dahl, L. K., M. Heine, and L. Tassinari. 1962a. Effects of chronic
excess salt ingestion: Evidence that genetic factors play an
important role in susceptibility to experimental hypertension.
J. Exptl. Med. 115: 1173-1190.
- Dahl, L. K., M. Heine, and L. Tassinari. 1962b. Role of genetic
factors in susceptibility to experimental hypertension due to
chronic excess salt ingestion. Nature 194: 480-482.
- Dahl, L. K., M. Heine, and L. Tassinari. 1963. Effects of chronic
excess salt ingestion: Role of genetic factors in both DOCA-salt
and renal hypertension. J. Exptl. Med. 118: 605-617.

- Dahl, L. K., M. Heine and L. Tassinari. 1964. Effects of chronic excess salt ingestion. Vascular reactivity in two strains of rats with opposite genetic susceptibility to experimental hypertension. Suppl. II, Circulation 29-30: 11-22.
- Dahl, L. K., M. Heine, and L. Tassinari. 1965. Effects of chronic excess salt ingestion. Further demonstration that genetic factors influence the development of hypertension: Evidence from experimental hypertension due to cortisone and to adrenal regeneration. J. Exptl. Med. 122: 533-545.
- Dahl, L. K., M. Heine, and K. Thompson. 1972. Genetic influence of renal homografts on blood pressure of rats from different strains. Proc. Soc. Exptl. Biol. Med. 140: 852-856.
- Dahl, L. K., M. Heine, and K. Thompson. 1974. Genetic influence of the kidneys on blood pressure: Evidence from chronic renal homograft in rats with opposite predisposition to hypertension. Circul. Res. 34: 94-101.
- Dahl, L. K., K. D. Knudsen, M. Heine, and G. Leitl. 1967. Effects of chronic excess salt ingestion: Genetic influence on the development of salt hypertension in parabiotic rats: Evidence for a humoral factor. J. Exptl. Med. 126: 687-699.
- Dahl, L. K., K. D. Knudsen, E. V. Ohanian, M. Muirhead, and R. Tuthill. 1975. Role of the gonads in hypertension. J. Exptl. Med. 142: 748-759.

- Dahl, L. K., and R. A. Love. 1954. Evidence for relationship between sodium (chloride) intake and human essential hypertension. Arch. Intern. Med. 94: 525-531.
- Dahl, L. K., and R. A. Love. 1957. Etiological role of sodium chloride intake in essential hypertension in humans. J. Amer. Med. Assoc. 164: 397-400.
- Dahl, L. K., and E. Schackow. 1964. Effects of chronic excess salt ingestion: Experimental hypertension in the rat. Canad. Med. Assoc. J. 90: 155-160.
- Dahl, L. K., L. Silver, R. W. Christie, and J. Genest. 1960. Adrenocortical function after prolonged salt restriction in hypertension. Nature 185: 110-111.
- Delisle, H. F., H. M. Brecht, J. M. Rojo-Ortega, A. Gattereau, and J. Genest. 1971. Effect of cadmium on heart content of norepinephrine in rats. Clinical Res. 19: 130. (Abstract)
- Dole, V. P., L. K. Dahl, G. C. Cotzias, H. A. Eder, and M. E. Krebs. 1950. Dietary treatment of hypertension. Clinical and metabolic studies of patients on the rice-fruit diet. J. Clinical Invest. 29: 1189-1206.
- Doyle, J. J., R. A. Bernhoft, and H. H. Sandstead. 1975. The effects of a low level of dietary cadmium on blood pressure, ^{24}Na , ^{42}K , and water retention in growing rats. J. Lab. Clinical Med. 86: 57-63.

- Foulke, J. D., W. V. Kessler, G. S. Born, S. M. Shaw, and R. J. Vetter. 1974. Effect of cadmium on sodium and potassium excretion and on action of hydrochlorothiazide in rats. J. Pharm. Sci. 63: 563-566.
- Free, A. H., and H. M. Free. 1972. Urinalysis critical discipline of clinical science. Rev. Clinic. Lab. Sci. 3: 481-531.
- Friedman, M., and S. C. Freed. 1949. Microphonic manometer for the indirect determination of systolic blood pressure in the rat. Proc. Soc. Exptl. Biol. Med. 70: 670-672.
- Friedman, R., and J. Iwai. 1976. Genetic predisposition and stress-induced hypertension. Science 193: 161-162.
- Grollman, A., T. R. Harrison, and J. R. Williams, Jr. 1940. Effect of various sterol derivatives on blood pressure of rat. J. Pharmacol. Exptl. Therap. 69: 149-155.
- Gunn, S. A., and T. C. Gould. 1957. Selective accumulation of ¹¹⁵Cd by cortex of rat kidney. Proc. Soc. Exptl. Biol. Med. 96: 820-823.
- Gunn, S. A., T. C. Gould, and W. A. D. Anderson. 1967. Specific response of mesenchymal tissue to cancerigenesis by cadmium. Arch. Pathol. 83: 493-499.
- Haber, E., T. Koerner, L. B. Page, B. Kliman, and A. Purnode. 1969. Application of a radioimmunoassay for angiotensin I to the

- physiologic measurements of plasma renin activity in normal human subjects. J. Clinic. Endocrinol. 29: 1349-1355.
- Iwai, J., L. K. Dahl, and K. D. Knudsen. 1973. Genetic influence on the renin-angiotensin system. Low renin activities in hypertension-prone rats. Circul. Res. 32: 678-684.
- Iwai, J., K. D. Knudsen, L. K. Dahl, M. Heine and G. Leitl. 1969. Genetic influence on the development of renal hypertension in parabiotic rats: Evidence for a humoral factor. J. Exptl. Med. 129: 507-522.
- Kägi, J. H. R., S. R. Himmelhoch, P. D. Whanger, J. L. Bethune, and B. L. Vallee. 1974. Equine hepatic and renal metallothioneins. J. Biol. Chem. 249: 3537-3542.
- Kägi, J. H. R., and B. L. Vallee. 1960. Metallothionein: A cadmium- and zinc-containing protein from equine renal cortex. J. Biol. Chem. 235: 3460-3465.
- Kanisawa, M., and H. A. Schroeder. 1969a. Life term studies on the effect of trace elements on spontaneous tumors in mice and rats. Cancer Res. 29: 892-895.
- Kanisawa, M., and H. A. Schroeder. 1969b. Renal arteriolar changes in hypertensive rats given cadmium in drinking water. Exptl. Molecul. Pathol. 10: 81-98.
- Kempner, W. 1944. Treatment of kidney disease and hypertensive vascular disease with rice diet. N. C. Med. J. 5: 125-133.

Kleinfeld, M., and E. Stein. 1968. Action of divalent cations on membrane potentials and contractility in rat atrium. *Amer. J. Physiol.* 215: 593-599.

Knudsen, K. D., and L. K. Dahl. 1966. Essential hypertension: Inborn error of sodium metabolism. *Postgrad. Med. J.* 42: 148-152.

Knudsen, K. D., J. Iwai, M. Heine, G. Leitl, and L. K. Dahl. 1969. Genetic influence on the development of renoprival hypertension in parabiotic rats: Evidence that a humoral hypertensinogenic factor is produced in kidney tissue of hypertension-prone rats. *J. Exptl. Med.* 130: 1353-1365.

Lener, J., and B. Bibr. 1970. Cadmium content in some food-stuffs in respect of its biological effects. *Int. J. Vitalstoffe Zivilisationskrankheiten* 15: 139-141.

Lener, J., and B. Bibr. 1971. Cadmium and hypertension. *Lancet* 1: 970.

Lewis, G. P., J. W. Jusko, L. L. Coughlin, and S. Hartz. 1972. Cadmium accumulation in man: Influence of smoking, occupation, alcoholic habit and disease. *J. Chronic Dis.* 25: 717-726.

Manthorpe, T. 1973. The effect on renal hypertension of subcutaneous isograft transplantation of renal medulla from normal or hypertensive rats. *Acta. Pathol. Microbiol. Scand. Section A.* 81: 725-733.

- Marsh, W. H., B. Fingerhut, and H. Miller. 1965. Automated and manual direct methods for the determination of blood urea. *Clinic. Chem.* 11: 624-627.
- Meneely, G. R., R. G. Tucker, W. J. Darby, and S. H. Auerbach. 1953. Chronic sodium chloride toxicity in Albino rat. II. Occurrence of hypertension and syndrome of edema and renal failure. *J. Exptl. Med.* 98: 71-80.
- Morgan, J. M. 1969. Tissue cadmium concentrations in man. *Arch. Intern. Med.* 123: 405-408.
- Nordberg, G. F. 1971. Effects of acute and chronic cadmium exposure on the testicles of mice. With special reference to protective effects of metallothionein. *Environ. Physiol.* 1: 171-187.
- Nordberg, G. F., M. Nordberg, M. Piscator, and O. Vesterberg. 1972. Separation of two forms of rabbit metallothionein by isoelectric focusing. *Biochem. J.* 126: 491-498.
- Nordberg, G. F., and M. Piscator. 1972. Influence of long-term cadmium exposure on urinary excretion of protein and cadmium in mice. *Environ. Physiol. Biochem.* 2: 37-49.
- Nordberg, G. F., M. Piscator, and M. Nordberg. 1971. On the distribution of cadmium in blood. *Acta Pharmacol. Toxicol.* 30: 289-295.

- Perry, H. M., Jr., and M. W. Erlanger. 1971. Hypertension and tissue metal levels after intraperitoneal cadmium, mercury, and zinc. *Amer. J. Physiol.* 220: 808-811.
- Perry, H. M., Jr., and M. W. Erlanger. 1973. Elevated circulating renin activity in rats following doses of cadmium known to induce hypertension. *J. Lab. Clinic. Med.* 82: 399-405.
- Perry, H. M., Jr., and M. W. Erlanger. 1974. Metal-induced hypertension following chronic feeding of low doses of cadmium and mercury. *J. Lab. Clinic. Med.* 83: 541-547.
- Perry, H. M., Jr., and M. W. Erlanger. 1975. Mechanism of cadmium-induced hypertension. P. 339-348. In D. D. Hemphill (Ed.), *Trace Substances in Environmental Health-IX*. Columbia, Missouri.
- Perry, H. M., Jr., M. W. Erlanger, A. Yunice, and E. F. Perry. 1967. Mechanisms of the acute hypertensive effect of intra-arterial cadmium and mercury in anesthetized rats. *J. Lab. Clinic. Med.* 70: 963-972.
- Perry, H. M., Jr., M. W. Erlanger, A. Yunice, E. Schoepfle, and E. F. Perry. 1970. Hypertension and tissue metal levels following intravenous cadmium, mercury, and zinc. *Amer. J. Physiol.* 219: 755-761.
- Perry, H. M., Jr., E. F. Perry, and J. E. Purifoy. 1971. Anti-natriuretic effect of intramuscular cadmium in rats. *Proc. Soc. Exptl. Biol. Med.* 136: 1240-1244.

- Perry, H. M., Jr., E. Schoepfle, and J. Bourgoignie. 1967. In vitro production and inhibition of aortic vasoconstriction by mercuric, cadmium and other metal ions. Proc. Soc. Exptl. Biol. Med. 124: 485-490.
- Perry, H. M., Jr., and H. A. Schroeder. 1955. Concentration of trace metals in urine of treated and untreated hypertensive patients compared with normal subjects. J. Lab. Clin. Med. 46: 936.
- Perry, H. M., Jr., and A. Yunice. 1965. Acute pressor effects of intra-arterial cadmium and mercuric ions in anesthetized rats. Proc. Soc. Exptl. Biol. Med. 120: 805-808.
- Porter, M. C., T. S. Miya, and W. F. Bousquet. 1974. Cadmium: Inability to induce hypertension in the rat. Toxicol. Appl. Pharmacol. 27: 692-695.
- Prodan, L. 1932. Cadmium poisoning: I. The history of cadmium poisoning and uses of cadmium. J. Indust. Health 14: 132-155.
- Pulido, P., J. H. R. Kägi, and B. Vallee. 1966. Isolation and some properties of human metallothionein. Biochemistry 5: 1768-1777.
- Rastogi, R. B., and R. L. Singhal. 1975. Effect of chronic cadmium treatment on rat adrenal catecholamines. Endocr. Res. Commun. 2: 87-94.

- Rush, R., L. Leon, and J. Turrell. 1971. Automated simultaneous^{140.}
cholesterol and triglyceride determination on the AutoAnalyzer II
instrument. P. 503-507. In Advances in Automated Analysis:
Technicon International Congress 1970 (Volume II): Clinical
Analysis. Thurman Associates, Miami, Florida.
- Scott, J., and F. Haddy. 1963. Local effects of Cd^{++} and Hg^{++}
on dog porelimb vascular bed. Feder. Proc. 22: 179. (Abstract)
- Schaikh, Z. A., and O. J. Lucis. 1970. Induction of cadmium-binding
protein. Feder. Proc. 29: 301. (Abstract)
- Schaikh, Z. A., and O. J. Lucis. 1972. Cadmium and zinc binding in
mammalian liver and kidneys. Arch. Environ. Health 24: 419-432.
- Schroeder, H. A. 1960. Relations between hardness of water and
death rates from certain chronic and degenerative diseases in
the United States. J. Chronic Dis. 12: 586-591.
- Schroeder, H. A. 1964a. Cadmium hypertension in rats. Amer. J.
Physiol. 207: 62-66.
- Schroeder, H. A. 1964b. Renal cadmium and essential hypertension.
J. Amer. Med. Assoc. 187: 122.
- Schroeder, H. A. 1965. Cadmium as a factor in hypertension. J.
Chronic Dis. 18: 647-656.
- Schroeder, H. A. 1967. Cadmium, chromium and essential hypertension.
Circulation 35: 570-582.

- 141.
- Schroeder, H. A. 1969. Editorial: The hard water. New England J. Med. 280: 836-838.
- Schroeder, H. A., J. T. Baker, N. M. Hansen, J. G. Size, and R. A. Wise. 1970. Vascular reactivity of rats altered by cadmium and a zinc chelate. Arch. Environ. Health 21: 609-614.
- Schroeder, H. A., and J. J. Balassa. 1961. Abnormal trace metals in man: Cadmium. J. Chronic Dis. 14: 236-258.
- Schroeder, H. A., and J. J. Balassa. 1965. Influence of chromium, cadmium and lead on rat aortic lipids and circulating cholesterol. Amer. J. Physiol. 209: 433-437.
- Schroeder, H. A., J. J. Balassa, and W. H. Vinton, Jr. 1965. Chromium, cadmium and lead in rats: Effects on life span, tumors and tissue levels. J. Nutrition 86: 51-66.
- Schroeder, H. A., and J. Buckman. 1967. Cadmium hypertension: Its reversal in rats by a zinc chelate. Arch. Environ. Health 14: 693-697.
- Schroeder, H. A., S. S. Kroll, J. W. Little, P. O. Livingston, and M. A. G. Myers. 1966. Hypertension in rats from injection of cadmium. Arch. Environ. Health 13: 788-789.
- Schroeder, H. A., A. P. Nason, and J. J. Balassa. 1967. Trace metals in rat tissues as influenced by calcium in water. J. Nutrition 93: 331-336.

Schroeder, H. A., A. P. Nason, and M. Mitchener. 1968. Action of a chelate of zinc on trace metals in hypertensive rats. Amer. J. Physiol. 214: 796-800.

Schroeder, H. A., A. P. Nason, R. E. Prior, J. B. Reed, and W. T. Haessler. 1968. Influence of cadmium on renal ischemic hypertension in rats. Amer. J. Physiol. 214: 469-474.

Schroeder, H. A., A. P. Nason, I. H. Tipton, and J. J. Balassa. 1967. Essential trace metals in man: Zinc. Relation to environmental cadmium. J. Chronic Dis. 20: 179-210.

Schroeder, H. A., and H. M. Perry, Jr. 1955. Antihypertensive effects of metal binding agents. J. Lab. Clinic. Med. 46: 416-422.

Schroeder, H. A., and W. H. Vinton, Jr. 1962. Hypertension induced in rats by small doses of cadmium. Amer. J. Physiol. 202: 515-518.

Schroeder, H. A., W. H. Vinton, Jr., and J. J. Balassa. 1963. Effect of chromium, cadmium and other trace metals on the growth and survival of mice. J. Nutrition 80: 39-47.

Slavin, S., G. E. Peterson, and P. C. Lindahl. 1975. Determination of heavy metals in meats by atomic absorption spectroscopy. Atomic Absorption Newsletter 14: 57-59.

Tipton, I. H., and M. J. Cook. 1963. Trace elements in human tissue. II. Adult subjects from the United States. Health Physics 9: 103-145.

Tipton, I. H., H. A. Schroeder, H. M. Perry, and M. J. Cook. 1965.

Trace elements in human tissue. III. Subjects from Africa,
the Near and Far East and Europe. Health Physics 11: 403-451.

Tsuchiya, K. 1969. Causation of ouch-ouch disease (Itai-Itai Byo).

An introductory. Part I. Nature of the disease. Part II.

Epidemiology and evaluation. Keio J. Med. 18: 181-194, 195-211.

Vander, A. J. 1962. Effect of cadmium on renal tubular sodium

transport. Amer. J. Physiol. 203: 1-5.

Webb, M. 1972a. Binding of cadmium ions by rat liver and kidney.

Biochem. Pharmacol. 21: 2751-2765.

Webb, M. 1972b. Protection by zinc against cadmium toxicity. Bio-

chem. Pharmacol. 21: 2767-2771.

Winge, D. R., and K. V. Rajagopalan. 1972. Purification and some

properties of cadmium-binding protein from rat liver. Arch. Biochem.

Biophys. 153: 755-762.