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EFFECT OF CADMIUM INDUCED HYPERTENSION ON SEVERAL RESPONSES OF AORTIC SMOOTH MUSCLE

by
Robert James Golden

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Environmental Health Sciences)
in The University of Michigan
1975

Doctoral Committee:

Professor Rolf Hartung, Chairman
Professor Gerald Abrams
Professor Herbert H. Cornish
Professor Harvey V. Sparks

DEDICATION

To my Father and Mother and Irene

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TABLE OF CONTENTS

DEDICATION ii

ACKNOWLEDGEMENTS iii

LIST OF TABLES vi

LIST OF FIGURES vii

INTRODUCTION 1

A. Acute and Chronic Cadmium Toxicity
B. Cadmium and Essential Hypertension
C. Sources of Cadmium Body Burden
D. Lack of Hypertension in Human Exposure
E. Cadmium Hypertension in Animals
F. Hypertension Models
G. Possible Defects in Hypertension
H. Structural and Mechanical Considerations
I. Calcium and Vascular Smooth Muscle
J. Similarities between Calcium and Cadmium
K. Research Approach

MATERIALS AND METHODS 23

I. Materials
   A. Chemicals
   B. Animals
II. Methods
   A. Animal Handling Procedures
   B. Administration of Cadmium
   C. Blood Pressure Measurement
   D. Preparation of Aortic Strips
   E. Mounting of Aortic Strips
   F. Procedure for Making Additions to Muscle Bath
   G. Determination of Tissue Cadmium
   H. Tissue Pathology

RESULTS 40

A. Production of Cadmium Hypertension
B. Effects of Chronic Exposure to Cadmium on Aortic Vascular Smooth Muscle

C. Length-Tension Studies
D. Response to Cadmium
E. Effects of $Na_2CaEDTA$
F. Acute Effects of Cadmium Ion
G. Effects of Cadmium under Anaerobic Conditions
H. Effects of Lanthanum on Vascular Smooth Muscle
I. Tissue Cadmium Levels
J. Half-life
K. Pathology

DISCUSSION 78

A. Control of Blood Pressure
B. Control of Vascular Smooth Muscle Tone
C. Production of Cadmium Hypertension
D. Effects of Several Agonists on Response of Aortic Strips
E. Responses of Aortic Strips to Cadmium Ion
F. Effects of Na2CaEDTA on Responses of Aortic Strips
G. Effects of Cadmium on Vascular Smooth Muscle under Anerobic Conditions
H. Tissue Cadmium Levels
I. Pathology

CONCLUSION 109

BIBLIOGRAPHY 112

LIST OF TABLES

Table		Page
I.	Blood Pressures of Rats Exposed to Cadmium Pre and Post Natally	43
II.	Organ Weights and Cadmium Levels of Exposed and Control Rats	68
III.	Total Liver and Kidney Cadmium Levels and Partial Computed Body Burdens	70
IV.	Decrease in Cadmium Levels after Stopping Cadmium Exposure	72

LIST OF FIGURES

Figure		Page
1.	Circuit Diagram for Photoelectric Blood Pressure Transducer	27
2.	Photoelectric Blood Pressure Transducer	29
3.	Typical Blood Pressure Recordings	31
4.	Comparison of Direct and Indirect Systolic Blood Pressure	33
5.	Jacketed Muscle Bath	36
6.	Effect of Norepinephrine on Contraction of Aortic Strips	44
7.	Effect of Serotonin on Contraction of Aortic Strips	46
8.	Effect of Potassium on Contraction of Aortic Strips	47
9.	Length-Tension Relationship between Control and Hypertensive Aortic Strips	50
10.	Effect of Cadmium on Control Aortic Strips	52
11.	Increase in Active Tension after Cadmium Contraction and Repeated Washout	53
12.	Effect of EDTA on Aortic Strips	55
13.	Redrawn Norepinephrine Curve after EDTA Correction	56
14.	Redrawn Serotonin Curve after EDTA Correction	58
15.	Redrawn Potassium Curve after EDTA Correction	59

LIST OF FIGURES

Figure		Page
16.	Separation of Contraction Phases by Cd and EDTA	61
17.	Effect of Lanthanum on Contraction of Aortic Strips	65
18.	Interactions between Cadmium and Lanthanum on Aortic Strips	66
19.	Illustrations of the Degrees of Observed Renal Damage	74
20.	Illustrations of the Degrees of Observed Renal Damage	75

INTRODUCTION

Today there is a growing awareness that many substances found in our environment have the potential to affect our health adversely. With continued population growth and industrialization worldwide, these potentially harmful substances will be constantly increasing. It is only in recent years that the menace to human health has even been recognized and even this growing awareness has not resulted in stemming the influx of these substances into the environment.

A. Acute and Chronic Cadmium Toxicity

Cadmium is such a substance and its toxicity has been known for many years. Its presence in the environment has increased many fold in recent years as new uses are found for this metal.

Acute responses after cadmium ingestion include such symptoms as severe nausea, salivation, vomiting, diarrhea and abdominal pains. When cadmium is inhaled as a fume it produces a severe pulmonary irritation with marked dyspnea, coughing, possible cyanosis and death (Browning, 1961). Chronic exposure to cadmium can produce such symptoms as fatigue, pallor, respiratory disturbance, weight loss as well as indications of renal

liver and bone impairment. The major pathological lesion seen in the lungs is emphysema with impaired respiratory function. Most cases of acute and even chronic cadmium toxicity are the result of an industrial exposure. Since 1945 the use of cadmium and cadmium compounds has increased sharply which has resulted in greatly increased environmental contamination. Cadmium is emitted into the air and water by mines, smelters, and industries which use cadmium (Friberg et al., 1971).

Non-industrial human exposure is therefore mainly through food and water with airborne cadmium contributing a lesser amount.

B. Cadmium and Essential Hypertension

Ever since Schroeder (1962) first described cadmium induced hypertension there has been a continuing controversy as to what role, if any, cadmium may play in human essential hypertension. There have been a number of positive correlations made between the presence of cadmium and hypertension.

An early observation made by Tipton (1963) was that there were large amounts of cadmium in the kidneys of adult Americans. She further noted that infants had no detectable renal cadmium and that adults from a relatively non-industrialized society, such as Africa, had considerably less renal cadmium than Americans. Cadmium levels

appear to increase with age but decline in old age (Livingston, 1971).

Hypertension is a disease which also becomes more evident with increasing age. When the kidneys of persons who died as a result of hypertension or hypertension related disease were examined for cadmium content it was discovered that they contained more cadmium than kidneys from persons who died from other causes (Schroeder, 1965; Flick et al., 1971; Schroeder, 1974). These same observations were made by Lener and Bibr (1971) when they measured renal cadmium levels in kidneys from hypertensive and normotensive patients. The levels in the hypertensive men were 36.1 $\pm$ 2.9 ug/g wet weight and the controls were 27.0 $\pm$ 3.1 ug/g wet weight. None of the subjects from either group had been exposed to cadmium. It must also be pointed out that this particular finding was not confirmed by Morgan (1969) who found that the only elevated renal cadmium levels were associated with various forms of neoplasia. These tumors were of a diffuse nature and in several cases involved the kidney and liver. There are a number of reports of an elevated urinary excretion of cadmium by persons with essential hypertension. Perry and Schroeder (1955) reported that there was up to a 40 fold increase in urinary cadmium in hypertension as compared to controls. This observation was confirmed by McKenzie and Kay (1973) in hypertensive women. While they

did not see a 40 fold increase the differences between the two groups were significant. There is also a report by Thind (1972) which indicates that patients with hypertension have higher plasma cadmium concentrations than normotensives.

There have also been several epidemiological studies done on the relationship between environmental cadmium and cardiovascular disease. Carroll (1966) found that the deaths associated with cardiovascular disease were highest in cities which had the highest levels of airborne cadmium. In Japan there was a higher incidence of hypertension (61.0 per cent) in farmers taking most of their calories in rice as compared to those whose diet was composed of more vegetables (41.3 per cent). The cadmium content of this particular rice was 123 ug/kg which is probably related to the cadmium containing phosphate fertilizer used on this crop. Persons who lived in areas of high rice consumption had high death rates from cerebral hemorrhage and hypertension (Takahashi et al., 1957).

C. Sources of Cadmium Body Burden

In general, most of the body burden of cadmium comes from dietary sources. Schroeder and Balassa (1961) found large amounts of cadmium in certain shellfish, mainly lobsters (13.2 ug/100g) and oysters (fresh 65 ug/100gm,

canned 140 ug/100g). They also found appreciable amounts in wheat (13.0 ug/100g), corn and oat grains (16.3 ug/100g). Cadmium finds its way into many grains because of its presence in certain types of phosphate fertilizers (commercial, 0-15-30 - 205 ug/100g, 20% super-phosphate, 230 ug/100g). Cadmium was also found in pork and beef kidney and liver as well as apple cider (50 ug/1), various vegetables and roasted peanuts. The United States Public Health Service has set a standard of 10 parts per billion (ppb) of cadmium in municipal water supplies. A number of cities in the United States have water which exceeds this standard by up to 3 times. Usually this increased water cadmium is associated with elevated cadmium in surrounding soil.

Cadmium is also found in tobacco and heavy smokers have significantly greater cadmium body burdens. A report by Lewis et al. (1972) states that the average body burden of an adult American male non-smoker was about 13 mg of cadmium. This is compared to a smoker's body burden of 30-40 mg. Cigarettes are known to contain appreciable amounts of cadmium (around 1.3 ug per cigarette) and about 20-40 per cent of this amount is estimated to be absorbed (Friberg et al., 1971). Recently Hirst et al. (1973) have shown a marked elevation of cadmium in emphysematous lungs. While they could not conclude that the cadmium caused the emphysema, most of the examined subjects had been heavy smokers.

D. Lack of Hypertension in Human Exposure

Even with the available evidence linking cadmium with hypertension there still remain a number of puzzling contradictions. Perhaps the hardest to explain is the fact that there does not appear to be a greater incidence of hypertension in persons who are chronically exposed to cadmium in an industrial setting. Probably the most common type of exposure in these situations is via the respiratory route. Depending on particle size and type of compound, cadmium is known to be absorbed through the respiratory route with a systemic retention of from 20-40 per cent. It is curious that cadmium has been reported to produce hypertension in various animals when administered orally, intraperitoneally, or intravenously but not by way of the lungs. The reasons for this are not known. Adding to the confusion of this issue is the Itai-itai disease (ouch-ouch) which was first described around 1955 in Japan. This is a disease which is partially the result of ingesting water and food containing cadmium. It is usually seen in highest incidence in postmenopausal women who have had multiple deliveries. The characteristic clinical manifestations of Itai-itai disease are lumbar pains, leg myalgia, bone deformation and extreme bone brittleness which leads to multiple fractures. Also seen are signs of renal involvement such as proteinura, glycosuria, amino-aciduria, as well as various pathological changes in the kidneys.

The bone disease seen is very similar to that found in osteomalacia (Friberg et al., 1971). Any mention of hypertension or any cardiovascular involvement in general is conspicuous by its absence.

E. Cadmium Hypertension in Animals

Despite the difficulties in deciding whether cadmium plays any part in human essential hypertension, its ability to produce hypertension in various laboratory animals is rather well established. Schroeder (1962) was able to produce hypertension in rats by feeding 5 parts per million (ppm) cadmium acetate in drinking water over a period of 12-24 months. There was a higher incidence of hypertension in females in this study. The strain of rat used in this study was the Long-Evans. When pathological examinations were made on the kidneys of these animals there were arterial and arteriolar lesions seen which were claimed to be indistinguishable from those seen in other forms of hypertension. Schroeder has since confirmed his own observations on a number of occasions (1965, 1967, 1968).

Hypertension has also been produced in animals when cadmium is administered intraperitoneally (IP). When rats are given 1 or 2 IP injections per week of cadmium citrate (1-2 mg/kg) they become hypertensive after a month (Schroeder and Buckman, 1967). These results were confirmed in rabbits by Thind et al. (1970) who gave weekly IP injections of 2 mg/kg cadmium acetate. The average

blood pressure produced after seven weeks of this treatment was 208 mm Hg and 100% of the treated animals became hypertensive. Thind et al. (1973) have also produced arterial hypertension in dogs by weekly IP injections of cadmium acetate at 2 mg/kg over a 9 month period. They failed to note any evidence of hematopoietic, liver or cardiac dysfunction in the hypertensive dogs. Cadmium is also an acute pressor agent when injected intravenously at low concentrations (0.1 - 0.4 mg/kg body weight) while higher concentrations (0.8 - 3.2 mg/kg) were found to depress blood pressure (Perry and Yunice, 1965).

In contrast to the above, there have been several investigators who have failed to produce hypertension with cadmium. Lener (1968) reported that no hypertension developed when female Wistar rats were given cadmium in drinking water (5ppm) for 16 months. Castenfors and Piscator (Friberg et al., 1971) were not able to induce hypertension in female Sprague-Dawley rats by thrice weekly injections (0.5 mg/kg cadmium chloride) for 6 months. Thus it appears that perhaps strain difference or other unknown factors may be determinants in the development of cadmium hypertension in experimental animals.

F. Animal Models for Hypertension Research

One of the problems in hypertension research is the unavailability of a completely suitable animal model for this disease. While there are a number of methods to produce hypertension in laboratory animals, all have drawbacks and none of them exactly mimics human essential hypertension. Most of the available methods are involved with the kidney since this organ is thought to be intimately involved with blood pressure control and regulation.

1. Goldblatt Hypertension

This is a type of hypertension which is produced in laboratory animals by either placing a constricting clamp on one renal artery with the contralateral kidney left intact (two-kidney type) or clamping one renal artery and removing the contralateral kidney (one-kidney Goldblatt hypertension). The hypertension produced is quite similar to human hypertension in many respects but the necessary surgical procedures make it a less than perfect model.

2. Increased Sodium Hypertension

Essential hypertension is generally characterized by alterations in sodium balance and extracellular fluid volume. If rats are maintained on a high sodium diet a persistent hypertension develops which is directly related to the amount of salt intake. These findings have been

confirmed in studies of human populations. If sodium intake is very low due to local customs or limited supply there is a virtual absence of hypertension. By the same token an excessively high salt intake can be linked to an increased frequency of hypertension.

3. Mineralocorticoid Hormone Hypertension

This type of hypertension is related to the sodium retention hypertension described above. Despite the fact that the specific mineralocorticoid, deoxycorticosterone, is a potent hypertensive agent, even large doses will produce little or no hypertension if the rat is maintained on a low salt diet. If rats are given deoxycorticosterone and maintained with 1% NaCl as the drinking fluid, up to 80% will be persistently hypertensive within 10 days. One problem with this type of hypertension is a noted difference among species in susceptibility to hypertension. Thus, an excess of either aldosterone or deoxycorticosterone, accompanied by the necessary sodium intake, will quickly produce hypertension in the rat, but not in the rabbit or the dog. There is some parallel with human hypertension since primary aldosteronism is a demonstrated cause of hypertension.

4. Spontaneously Hypertensive Rats

Recently a strain of Wistar rat has been inbred which will spontaneously become hypertensive. These animals were developed by Okamoto and Aoki (1963) and have demonstrated close parallels with human essential hypertension--significant age-related blood pressure elevation, characteristic pathologic renal changes, as well as other cardiovascular and cerebrovascular complications. These animals develop hypertension after about 80 days but vascular lesions usually do not appear until 180 days after birth (Okamoto et al., 1964). While this model is perhaps the best developed to date, it is also not without problems. Since this is a highly inbred strain, there is considerable disagreement as to what constitutes a suitable control. Usually Wistar rats from the same strain as the spontaneously hypertensive rats are used as controls.

5. Cadmium Hypertension

While cadmium is known to induce a persistent and severe hypertension in several species of laboratory animals, it has not achieved much popularity in the laboratory study of hypertension. As in the other forms of experimental hypertension this type also bears some similarity to the human disease. It is age related and females appear more susceptible than males. Cadmium hypertension in rats also duplicates the human disease by increased

mortality, renal arteriolar sclerosis and an increase in the severity of atherosclerosis (Schroeder, 1974). There has not been enough critical study of cadmium hypertension to determine if it deserves to be included as a possible model of human hypertension. Hopefully the results of this investigation will be a start toward resolving this issue.

G. Possible Defects in Hypertension

Hypertension is an extremely complicated disease and the search for a single causative entity has been largely abandoned. Current investigation has focused in many areas of possible physiological or biochemical involvement. Abnormalities relating to peripheral resistance, cardiac output, renin and aldosterone production, blood-plasma volume and sympathetic nervous system activity are all under current study.

Since the scope of this investigation will be limited to studies of changes in vascular smooth muscle, only this aspect of the possible alterations mentioned above will be briefly reviewed. Even in this limited area there exists an overwhelming amount of data complete with contradictions and confusion.

Reactivity of vascular smooth muscle can be conveniently studied *in vitro* by using helically cut spiral strips prepared according to the method of Furchgott

(1963). An artery is removed and cut in such a way that the circular muscle will fall on the longitudinal axis of a small strip. Muscle contractions which would normally cause a constriction *in vivo* then produce a shortening of the vascular strip which can then be measured with sensitive force transducers. Several strips prepared in this way can then be compared in a single muscle bath.

Observations made with this technique have indicated that vascular smooth muscle strips from hypertensive animals often behaved differently than normotensive strips. Redleaf and Tobian (1958) reported that rats made hypertensive with deoxycorticosterone acetate (DCA) as well as renal hypertensive rats developed either the same or less contractile force in aortic strips preparations when exposed to norepinephrine than normotensive strips. This same observation was also made by Shibata *et al.* (1973) on aortic strips from spontaneously hypertensive rats. These observations were confirmed by Spector *et al.* (1969) who noted a decreased responsiveness to norepinephrine, serotonin and KCl. Femoral arteries from renal hypertensive rats were less responsive to norepinephrine than controls but had a lower threshold to norepinephrine than controls (Bandick and Sparks, 1970). This contridicts the observations of Gordon and Nogueria (1962) who reported that aortic strips from renal hypertensive

rats developed more active tension than controls when exposed to norepinephrine. The above observations are balanced by those of Clineschmidt et al. (1970), who reported no difference between aortic strips from spontaneously hypertensive rats or controls to the effects of norephinephrine. When Thind et al. (1969) compared the aortic reactivity from cadmium hypertensive rabbits with controls he noted no difference between them to the effects of epinephrine but noted a decreased responsiveness in the hypertensive strips to the effects of angiotensin. The apparent or real contradictions in these observations make it difficult to draw conclusions about this aspect of vascular smooth muscle reactivity.

The suggestion has been made by some that hypertensive vessels may have a lower threshold to the effects of norepinephrine. This implies that such vessels would respond in vivo to concentrations of norepinephrine which normal vessels would not detect. There is some evidence that this may be true. Field et al. (1972) report that aortic strips form renal hypertensive and spontaneously hypertensive rats produce significantly more tension at low concentrations of norephinephrine (1×10^{-10} -3×10^{-10} M) than controls. A similar observation was made by Bandick and Sparks (1970) on aortic strips from renal hypertensive rats.

H. Structural and Mechanical Considerations

There is wide agreement that hypertension of any etiology produces physical changes in the arteries. These changes are usually described as an increased thickness of the arterial wall with a corresponding decrease in the size of the lumen. Assuming cardiac output remains constant, arteriolar changes such as this could easily account for an increased peripheral resistance and therefore elevated blood pressure. Bandick and Sparks (1970) concluded that the increased stiffness seen in the aortas from renal hypertensive rats was the result of smooth muscle hypertrophy. This increase in smooth muscle could possibly explain increased responsiveness to various agonists. What is not clear, however, is whether these changes are the cause of the hypertension or the result of it.

Another vascular alteration which occurs in hypertension is an increased water and sodium content in arterial walls. It is not known if excess water is drawn into the tissue by the elevated sodium levels which are often associated with hypertensive conditions (Tobian and Binion, 1952). Harris (1970) has reported on the sodium binding capacity of the mucopolysaccharides found in arterial

walls. These mucopolysaccharides are also known to increase in the arteries in various forms of experimental as well as human hypertension (Manley, 1965). This could result in an increased sodium content which may be associated with altered vascular reactivity. In any case the increased content of water and solids can cause narrowing of the lumen as well as decreased elasticity and compliance of the artery, all of which can contribute to increased vascular resistance (Tobian, 1974).

This nonmyogenic narrowing of arterioles has also been observed by Folkow (1971) in both human essential hypertension and in the spontaneously hypertensive rat. The claim is made that the basal vascular tone is the same in hypertensive and normotensive subjects but the resistance is increased due to the non-myogenic arteriolar narrowing. When sympathetic stimulation occurs in these vessles there is an even greater increase in resistance when compared to normotensive vessels.

I. Calcium and Vascular Smooth Muscle

Perhaps the single most important controlling factor which regulates the functioning of vascular smooth muscle is ionic calcium. The contractile protein of smooth muscle, like skeletal muscle, is actomyosin. This protein which derives its energy for contraction from ATP by virtue of its ATPase activity is normally inhibited by

another protein complex--troponin-tropomyosin. The presence of free ionic calcium depresses the inhibition of the troponin-tropomyosin and allows the ATPase activity of the actomyosin to release free energy for contraction (Somlyo and Somlyo, 1968; Murray and Weber, 1974).

Ionic calcium, therefore, plays a central role in maintenance of tone, contraction and relaxation of vascular smooth muscle. Unlike skeletal muscle, vascular smooth muscle does not have a highly developed sarcoplasmic reticulum and therefore the plasma membrane is thought to play the major role in regulating the distribution of ionic calcium between the intracellular and extracellular compartments (Seidel and Bohr, 1971).

Generally, ionic calcium is believed to gain access to the contractile proteins from two sources. The calcium pool closest to the contractile proteins is intracellular. This is normally sequestered or tightly bound to the inside of the plasma membrane or some other intracellular component but in any case is not available in an ionic form to the contractile proteins. A contractile stimulus somehow causes the release of ionic calcium which becomes available to initiate the excitation-contraction coupling process. The amount of free energy released and consequently the magnitude of the contraction is directly related to the amount of ionic calcium which reaches the contractile proteins (Bohr and Sitrin, 1970). The other source of calcium is extracellular where the concentration

of calcium may be up to 1000 times that of the intracellular calcium concentration. In order to maintain this tremendous concentration gradient between the inside and outside of the membrane, an active pump in the membrane which extrudes calcium has been postulated. While such a pump has yet to be identified it seems unlikely that the gradient could be maintained in any other way. A subcellular microsomal fraction of vascular smooth muscle has been identified by Fitzpatrick et al. (1972) which takes up calcium in the presence of magnesium and ATP. This may well be part of the calcium extrusion system which maintains the gradient as well as regulates vascular tone.

That extracellular calcium plays an important role in smooth muscle contraction is illustrated by two observations. First, the response of vascular smooth muscle to norepinephrine, for example, is directly related to the extracellular calcium concentration. If the concentration is high, the response is augmented, and if lowered, the response is diminished (Godfraind et al., 1972). Second are the effects of agonists on vascular smooth muscle in a calcium free medium. First it must be mentioned that vascular smooth muscle exhibits a two component response to norepinephrine and other agonists. The fast or phasic component is due to release of intracellular calcium to the contractile apparatus and the slow or tonic component is produced by the influx of extracellular calcium

(Steinsland et al., 1972). When strips of vascular smooth muscle are stimulated with norepinephrine in a calcium free medium the slow component is abolished. The slow component can be completely recovered if calcium is added to the medium at the peak of the fast response (Sitrin and Bohr, 1971). Further stimulation with norepinephrine results in a steadily diminishing contractile response of the fast component.

It appears that the smooth muscle cell membrane plays a vital role in regulating calcium flux and therefore vascular tone as well as the differing responses to numerous agonists. Somlyo and Somlyo (1968) postulate that the amount of calcium bound to the membrane regulates the permeability of the membrane to ionic calcium as well as other substances. According to this hypothesis potassium depolarization causes a release of membrane-bound calcium as well as an increased permeability to extracellular calcium. This idea is partially supported by the work of vanBreeman and Lesser (1971) who demonstrated that lanthanum which binds more strongly to calcium binding sites than calcium, will prevent a high potassium depolarization contraction. Norephinephrine on the other hand, may eliminate the stabilizing effect that calcium has on the membrane with or without releasing it from these sites. This is indicated by the fact that lanthanum does not block the initial or fast component of a norepinephrine induced contraction.

J. Similarities between Calcium and Cadmium

There are certain striking similarities between calcium and cadmium. Both are divalent cations and share other similarities as listed below (Lakshminarayanaiah, 1969; Nançollas, 1966).

	Ionic crystal radii(Å)	Ionic entropy S^o (ion) (cal. deg. $^{-1}$)
Ca^{+2}	0.99	-13.2
Cd^{+2}	0.97	-14.6

IONIC HYDRATION NUMBERS

Method of electrolytic transport (D_2O)	
Ca^{+2}	29.0
Cd^{+2}	39.0
Method of ionic mobility (H^+=1)	
Ca^{+2}	10.0
Cd^{+2}	11.0
Method of Raoult's law (Cl^-=3)	
Ca^{+2}	10.5
Cd^{+2}	15.0

Beside the purely physical similarities between the two molecules, there is some evidence of a competitive effect for certain biological sites. Hajdu *et al.* (1971) have reported that cadmium would replace calcium at binding sites involved in excitation-contraction coupling.

They further noted that cadmium was bound more strongly to these sites than calcium. While these observations were made on rat diaphragm preparations, they do indicate the strong possibility of a similar effect in vascular smooth muscle.

K. Research Approach

Despite the fact that cadmium has been shown to induce hypertension in a variety of laboratory animals, the mechanism of its action has not been elucidated. Furthermore, there is still considerable doubt as to what role cadmium may play in human essential hypertension. In light of these uncertainties, a series of experiments was undertaken to characterize further the effects of cadmium on vascular smooth muscle. It would be very helpful, for example, to know whether cadmium alters vascular smooth muscle in a completely unique way or if the alterations are consistent with other forms of experimental hypertension. There are many reports describing cadmium-induced hypertension in laboratory animals and continued speculation upon its role in the human disease. Nevertheless, the mechanisms by which cadmium produces its effects are poorly understood.

To this end a research program was undertaken which would attempt to answer two fundamental questions. First, by what basic mechanism is cadmium able to produce

elevated blood pressures in laboratory animals? Second, how does this mechanism of action compare or fit in with existing models of experimental hypertension?

Hypertension was first produced in several strains of rats to determine if one would be a more suitable experimental subject. Vascular reactivity was compared using the previously described isolated strip technique. The aorta was chosen as the vascular smooth muscle to study, despite the fact that it is not important in maintaining peripheral resistance; the reasons for this choice were: 1. the thoracic aorta by virtue of its larger size would be easier to prepare for the strip technique than a smaller vessel, 2. there already existed a large body of information on the various responses of aortic strips from normotensive and hypertensive rats.

Reactivity to the agonists norepinephrine, serotonin and potassium chloride was determined for chronic cadmium hypertensive and normotensive aortic strips. The direct effects of cadmium ions were also studied on both chronically and acutely exposed vascular smooth muscle. An attempt was also made to correlate <u>in vivo</u> effects of chelation on cadmium hypertension with chelating agents on the aortic strip preparations. Cadmium concentrations in kidney, liver and aorta were also determined.

MATERIALS AND METHODS

I. Materials

A. Chemicals

Cadmium acetate and cadmium chloride were purchased from Matheson, Coleman, and Bell. Sodium chloride, potassium chloride, potassium phosphate, magnesium sulfate, sodium bicarbonate, dextrose and sucrose were obtained from Mallinckrodt Chemical Works or J. T. Baker Chemical Co. The $Na_2CaEDTA$, serotonin and norepinephrine were purchased from Sigma Chemical Company. Lanthanum chloride was purchased from Alfa Products. All other chemicals used and not specifically listed here were reagent grade or of the highest purity available.

B. Animals

The animals first used in this study were male Sprague-Dawley albino rats. These animals were purchased from Spartan Research Animals, Inc., Haslett, Michigan. When it was decided to test the susceptibility of different strains of rats to the effects of cadmium, two additional kinds of rats were obtained. Breeding pairs of Wistar rats were purchased from Carworth Midwest Facility, Portage, Michigan. Breeding pairs of BLU: LE BR

Long-Evans descent hooded rats were purchased from Blue Spruce Farms, Inc., Altamont, New York.

II. Methods

A. Animal Handling Procedures

Upon arrival the Sprague-Dawley rats were pair housed in stainless steel cages and fed a diet of Teklad Rat Chow and water ad libitum. The breeding pairs were placed in plastic breeding cages with the same chow as above. As soon as the young were old enough to be separated from the mother they were also pair housed in stainless steel cages.

B. Administration of Cadmium

Cadmium hypertension can be produced by either the IP or oral route of administration. Initially the IP route was chosen because the hypertension will develop faster. Cadmium acetate was dissolved in 0.9% sodium chloride and injected intraperitoneally (IP) at a dose of either 2 or 4 mg/kg. Controls received 0.9% NaCl. Each animal was injected once per week and the blood pressures monitored every few weeks. Higher doses of 6 and 8 mg/kg were tried, but the animals could not tolerate this dose and died. Rats of both sexes were used in this study to determine if there was a sex difference in cadmium induced hypertension.

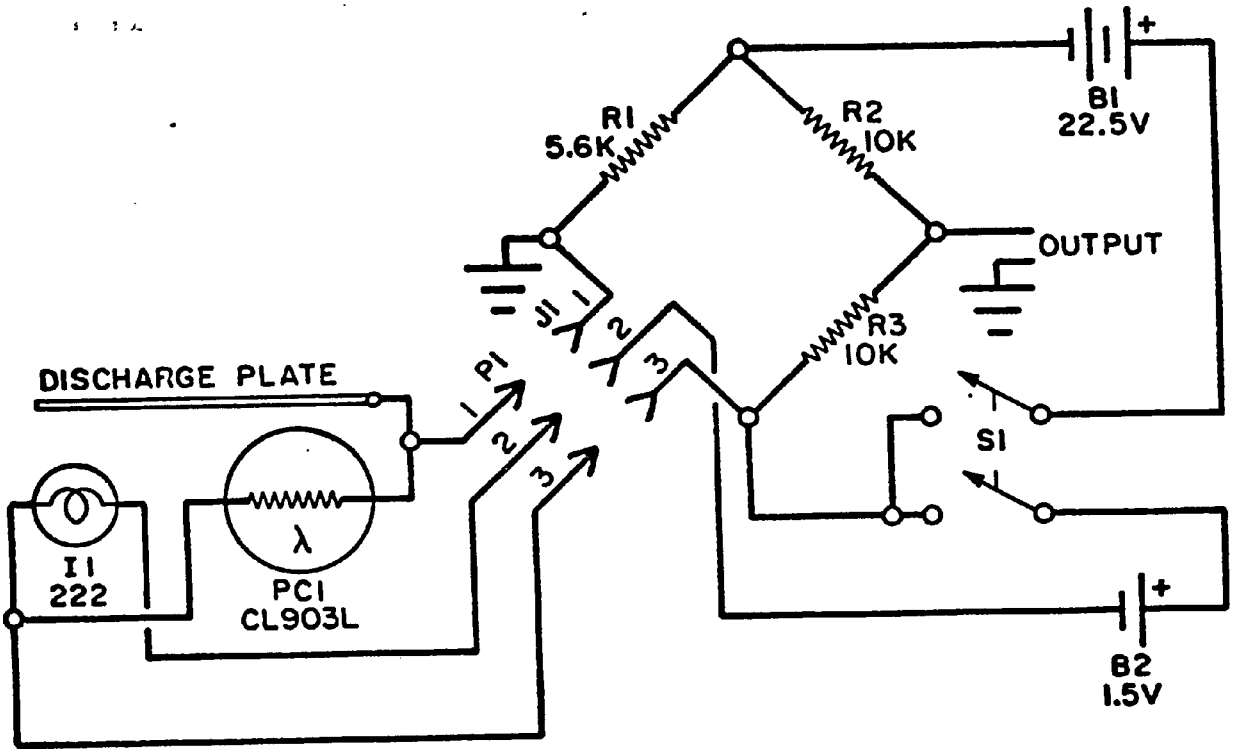
The IP route of administration of cadmium was successful in producing hypertension in a large number of animals. There was a problem, however, in that all of the cadmium injected animals developed dense intra-abdominal adhesions. Some of these adhesions were so extensive that the viscera were fused together into a single mass. While these adhesions did not impinge on the thoracic aorta, it was felt that an animal which was compromised in this way was not a suitable experimental subject.

At this point the decision was made to continue the study using the oral route of administration. While animals exposed to cadmium by the oral route take longer to develop hypertension there would be no complicating adhesions. Schroeder (1963) administered $CdCl_2$ in the drinking water at a concentration of 5 parts per million (ppm). This produced hypertension in a large percentage of animals but it took 18-24 months to develop. It was therefore decided to use a higher concentration, since only about 5% of an oral dose is absorbed (Moore *et al.*; Friberg *et al.*, 1971). At cadmium concentrations above 250 ppm the rats will not drink the water and will die from dehydration. The concentration finally adopted was 100 ppm of cadmium chloride in the drinking water *ad libitum*. The blood pressure of these animals was monitered approximately once per month.

C. Blood Pressure Measurement

A traditional method to obtain the blood pressure of a rat is by the use of the tail cuff with a distal pressure sensitive device. Usually this device is a piezoelectric transducer which receives a signal from a pneumatic sensor. This sensor is secured to the tail distal to the cuff. Commonly a rat is anesthetized before this procedure since any movement will produce an artifact in the output signal. Perhaps the greatest drawback of this method is the fact that the animal must be heated until the tail artery dilates enough so a pulsatile signal can be detected. While this method can be used on single animals it is not easily adapted to screening a large number of animals on a long term basis. For this reason a number of other methods were investigated to determine if there were any which would be more efficient for the purposes stated above.

The method which was finally adopted utilized a photoelectric pulse transducer (Swales and Tange, 1970). The circut diagram for this device is shown in Figure 1. This device has the added advantage that the hind foot can be used which eliminates the need to warm the animal. Also the anesthetic could be ether instead of Sodium Pentobarbital since the animal only had to be unconscious for a few minutes.



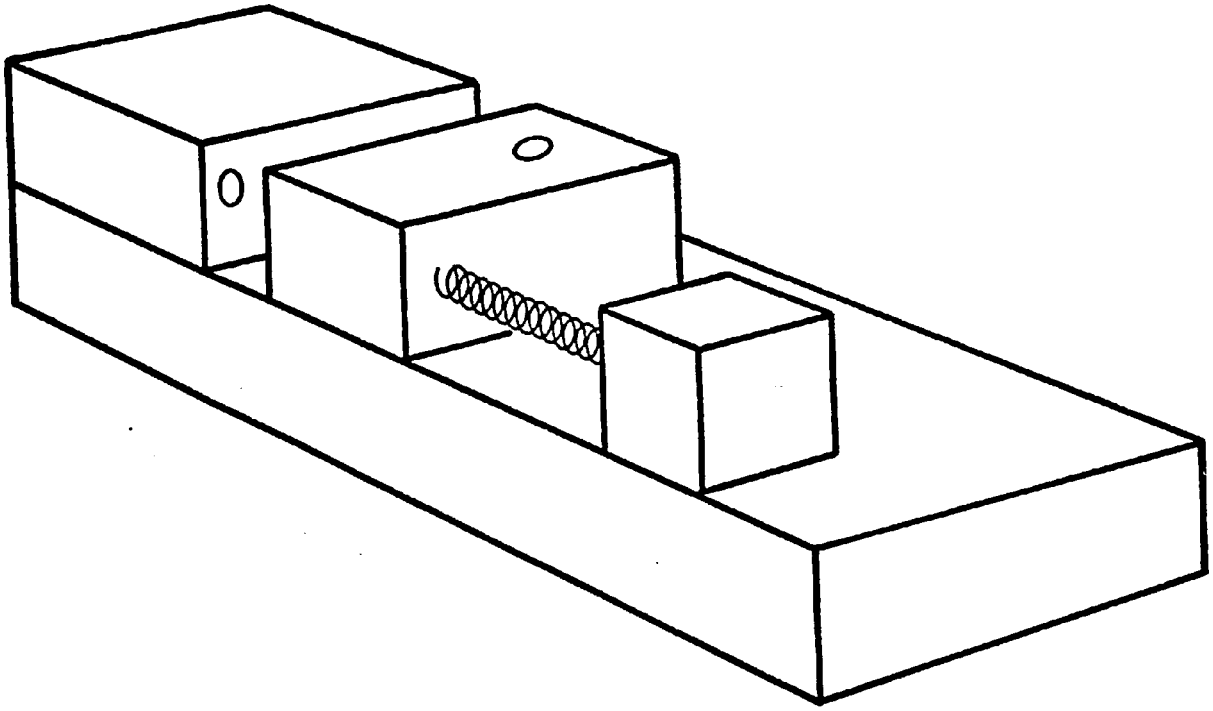
CIRCUIT DIAGRAM FOR
PHOTOELECTRIC BLOOD PRESSURE TRANSDUCER

Figure 1. The photoelectric transducer is a simple bridge with the photocell PC1 as the variable arm. The plate bleeds off static electricity. The output cable is a shielded single-lead microphone cable. This cable is connected to both the oscilloscope and the Physiograph, Devine (1968).

The photoelectric device takes advantage of the fact that tissues are relatively transparent to the red part of the light spectrum while the blood is not. During each systolic beat of the heart there is an increased amount of blood in any given artery. When the hind foot is placed between a light source and a photoelectric cell, less light will reach the photocell during each pressure pulse. This decrease in light to the photocell causes it to change its resistance which can be amplified and recorded. When an occlusive cuff is placed proximal to the photocell transducer the pressure can be raised and slowly lowered until the signal is detected. The systolic pressure is the cuff pressure where this signal is first detected.

Light ether anesthesia was used on each animal. The entire procedure takes two or three minutes. The procedure is as follows: As soon as the animal loses consciousness it is taken from the ether jar and placed on a flat surface. The cuff (20 mm width) is put on the thigh and the foot positioned in the transducer shown in Figure 2. The room lights must be turned off since the photocell is so sensitive that it can pick up some 60 cycle interference. The signal is first displayed on an oscilloscope (Tektronix Type 564B Storage Oscilloscope with Auto-Erase) until the pattern is constant. The signal is split from the oscilloscope to one channel of a six channel Physiograph so it can be recorded. The cuff pressure is recorded on another channel. As the cuff

PHOTOELECTRIC BLOOD PRESSURE TRANSDUCER



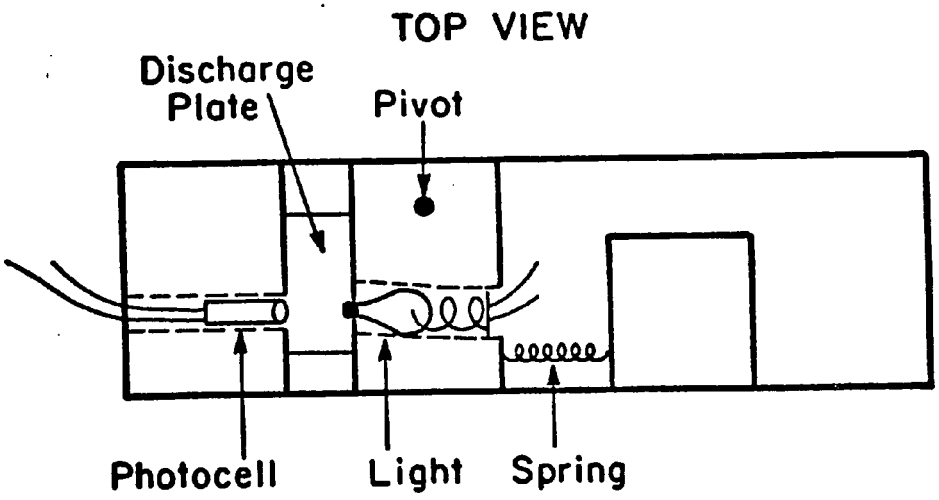


Figure 2. The transducer is made of wood. The block containing the light is free to move about a pivot which allows a snug fit around the foot.

pressure is increased the signal from the transducer stops and reappears at some point as the pressure is released. Figure 3 illustrates some typical blood pressure determinations. Three or four determinations were made on each animal and were remarkably consistent. Occasionally the animal's respirations would be superimposed over the arterial signal but these do not interfere with the blood pressure determination.

In order to use an indirect method for blood pressure determination it is necessary to know that there is a high correlation between the indirect reading and the acutal direct pressure. There are many factors which must be taken into account to make this comparison. State of anesthesia, type of anesthetic, type of indirect transducer, placement of transducer, heating, restraint, cuff width, are but a few of the many variables which can affect an indirect blood pressure determination. A number of investigators, Frohlich et al., 1972, Patten et al., 1971, Bunag et al., 1971, and Bunag, 1973, as well as others have concluded that the indirect blood pressure is a valid indicator of direct blood pressure. It must be remembered that the indirect method will give only the systolic pressure and not the diastolic pressure. This is not meant to imply that the indirect methods are without problems. The width of the cuff, for example, can cause the observed pressure to fluctuate above or below the actual arterial pressure (Burch and

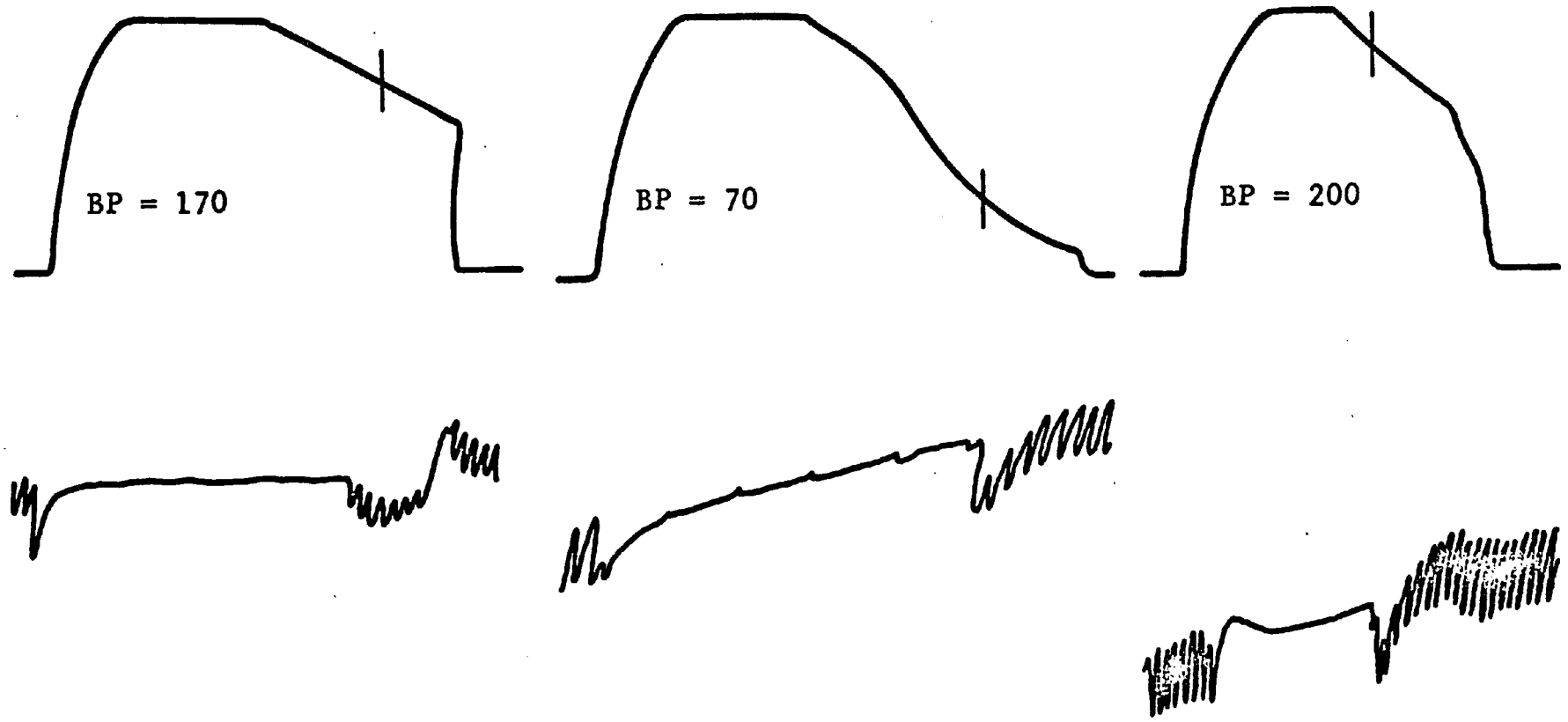


Figure 3. Some typical blood pressure recordings made using the photoelectric transducer with an occlusive cuff. The top line is the pressure in the cuff and the bottom tracing is the signal from the transducer.

Shewey, 1973). In general, anesthetics will all lower the blood pressure in both direct and indirect methods. Since warming of the animals is not necessary for blood pressure measurement on the hind foot it was not a complicating factor.

To compare the hind leg photoelectric method with the direct arterial pressure a number of rats were cannulated in the abdominal aorta. The direct blood pressure was obtained under ether anesthesia with a Narco-Bio Systems Linear-Core Pressure Transducer which was connected to a Six-Channel Physiograph. This was done only minutes after the indirect pressure was measured on the hind foot. A number of animals were also given an appropriate dose of epinephrine (IM and IP) so blood pressures above the normal range could be compared by both methods. The correlation coeficient between the two methods had a value of .9685 which shows excellent agreement between the two methods. This is further illustrated by Figure 4 which is a scatter diagram comparing the direct and the indirect methods.

D. Preparation of Aortic Strips

A rat was decapitated and the thoracic aorta quickly removed and placed in a petri dish of cold (4° C) physiological salt solution (PSS). The composition of the buffer was as follows:

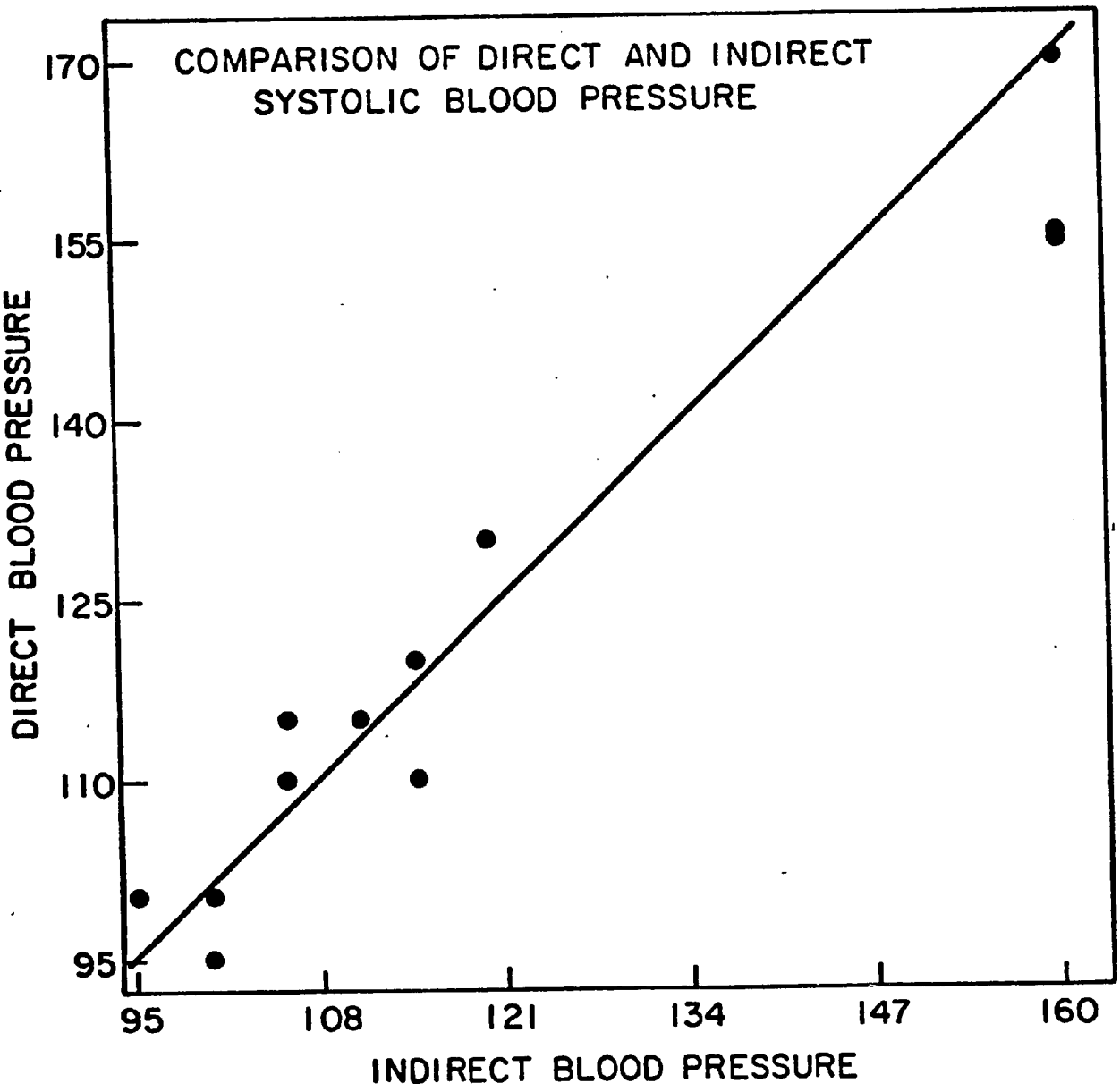

COMPARISON OF DIRECT AND INDIRECT
SYSTOLIC BLOOD PRESSURE
DIRECT BLOOD PRESSURE
170
155
140
125
110
95
95
108
121
134
147
160
INDIRECT BLOOD PRESSURE

Figure 4.

	mM/l
NaCl	119.0
K Cl	4.7
KH_2PO_4	1.18
$MgSO_4 - 7H_2O$	1.17
$NaHCO_3$	14.9
Dextrose	5.5
Sucrose	50.0
$CaCl_2$	1.6

The petri dish had a layer of paraffin in the bottom so the tissue could be anchored with pins for preparation. A blunt 18 gauge needle was used to rinse the blood gently from the segment of aorta. Fat and connective tissue were then carefully cut away from the aorta. The aorta was then placed over a bent insect pin and the petri dish put under a Bausch and Lomb Disecting Microscope. The aorta was cut into a helical strip at an angle of approximately 60° to the long axis. Aortic strips were always from 1 to 2 mm in width. The cut strip was then pinned under no tension so it could be tied into appropriate lengths. 4-0 silk was used to tie loops to each strip so it could be mounted in the muscle bath and attached to the force transducers. Each aortic strip was approximately 10 mm in length. Four strips could be prepared from one aorta. Usually strips could be stored 24 hours in PSS at 4° C.

When the studies with lanthanum were done a different buffer solution had to be used. Lanthanum will form insoluble precipitates with PO_4^{-2} and CO_3^{-2} so these must be replaced with a Tris buffer. The solution for these experiments had the following composition in millimoles per liter:

	mM/1
NaCl	130.0
K Cl	6.0
$CaCl_2$	1.6
$MgCl_2$	1.2
Dextrose	5.5
Sucrose	60.0
Tris Buffer (100 ml/1)	5.0

This buffer solution was adjusted to a pH of 7.4 and aerated with 100% O_2.

E. Mounting of Aortic Strips

Two prepared strips were mounted in a 10 ml jacketed muscle bath as illustrated in Figure 5. One end of each strip was secured to a rigid glass hook and the other to Narco Bio-Systems photoelectric force transducers. Contractions were measured on a Physiograph Six. The temperature of of the bath was maintained at 37°C. The physiological salt solution (PSS) was aerated with 95% O_2 and 5% CO_2 and the pH was 7.4.

The transducers and Physiograph were calibrated before each experiment by hanging known weights on the transducers and adjusting the pen deflection on the Physiograph. The calibration for all experiments was such that 1.0 mm of pen deflection was equivalent to 50 mg of tension. The aortic strips were placed under 1.0 g resting tension and allowed to equilibrate for at least 60 minutes before an experiment was started. During the

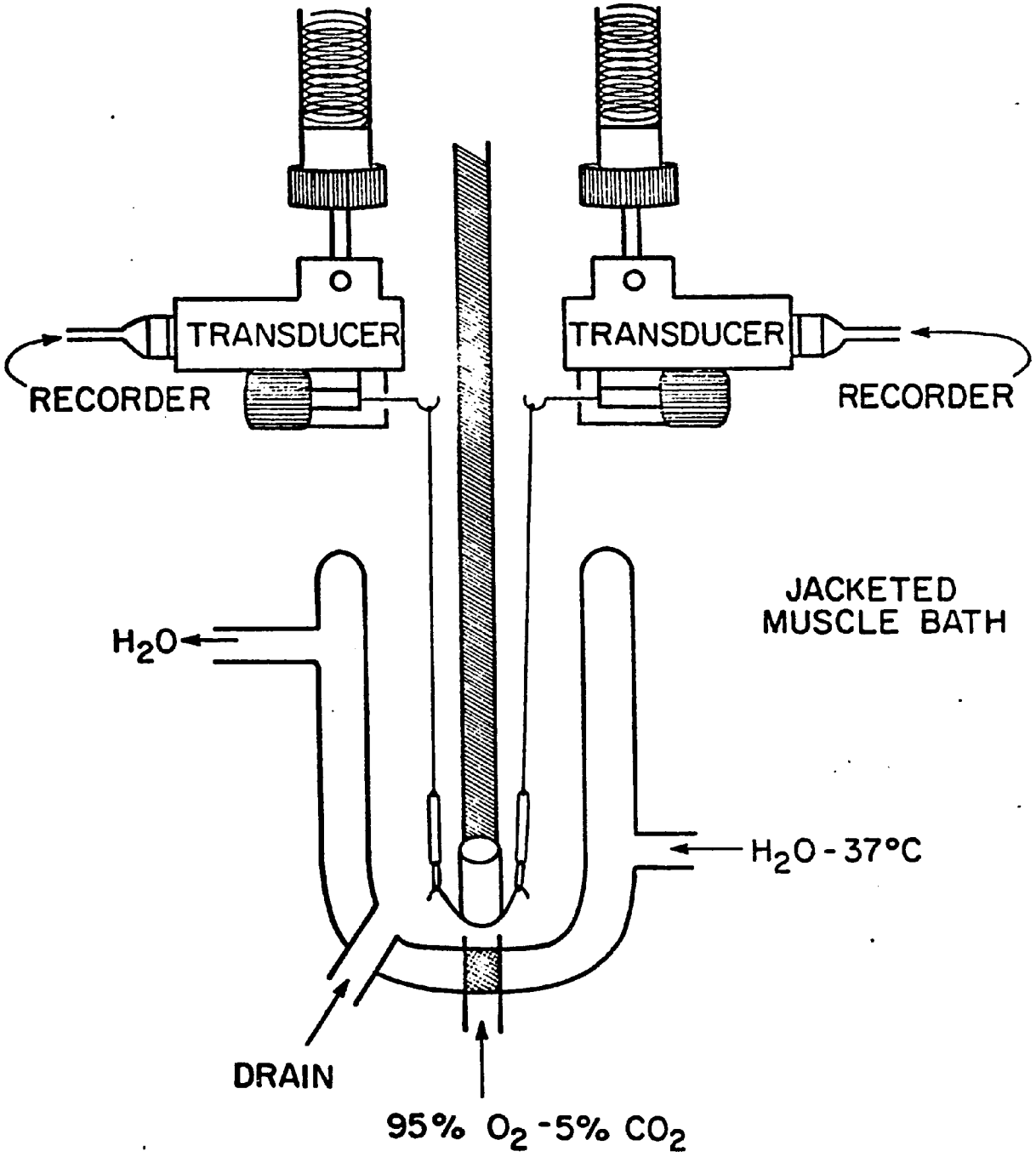


Figure 5. This illustrates the jacketed muscle bath along with the force transducers and the support rod. Two aortic strips are also shown.

equilibration period the PSS was changed every 15-20 minutes to prevent the possible accumulation of metabolic end products which have been claimed to affect subsequent responses (Altura, 1970). This method makes it possible to compare an aortic strip from a control animal with a strip from an experimental animal under the same conditions. Throughout the course of research no animal was used as a cadmium hypertensive animal unless its systolic blood pressure was 170 or higher. In this way the problem of borderline hypertension was avoided.

F. Procedure for Making Additions to Muscle Bath

The various drugs and agonists used in the course of this investigation were either prepared with PSS or added to the bath in 0.1 ml amounts (1% of total volume). At all times the volume of the bath was maintained at 10 ml.

G. Determination of Tissue Cadmium

After the entire organ had been weighed, a small piece was cut and weighted and placed in a 2 oz. Nalgene polyethylene bottle. To the tissue was added an appropriate (1-2 ml) amount of nitric acid-perchloric acid solution (7:3) and the bottle tightly capped and placed in a hot tap water bath for 12-18 hours. A combination of the acid, temperature and pressure completely digests the tissue in this time (Adrian, 1971).

After this process was completed the entire amount of digest was transferred to a 100 ml volumetric flask. The plastic bottle was rinsed twice with distilled water and these rinses were also added to the flask. 10 ml of an ammonium-citrate buffer (pH=8.4) solution were added to the flask followed by 6 drops of 0.1% phenol red indicator. Sufficient concentrated ammonia was then added until the indicator turned red. The cadmium was then chelated by the addition of 2 ml of sodium diethyldithiocarbamate (NaDDTC). The flasks were swirled and allowed to stand for 30 minutes to allow complete chelation of cadmium with NaDDTC. Methylisobutylketone (MIBK) was then added (5ml) and each flask was shaken vigorously for 30 seconds to extract the NaDDTC into the MIBK. Distilled water was added down the side of the flask to bring the organic phase into the neck of the flask (Hartung, 1973). Both standards and blanks were prepared in the manner just described.

Cadmium determination was carried out on a Perkin-Elmer 403 Atomic Absorption Spectrophotometer. The zero signal was set while burning water saturated MIBK. This was also burned between samples. The organic phase in the neck of each flask was aspirated into the flame. The cadmium in each sample was calculated after subtracting the amount of the blank.

H. Tissue Pathology

Samples of liver, kidney and aorta were prepared for pathological examination by the Department of Pathology of The University of Michigan Medical School. All tissues were fixed in 10% formalin and stained with hematoxylin and eosin (H and E).

RESULTS

A. Production of Cadmium Hypertension

Cadmium is able to produce hypertension when administered by either the intraperitoneal (IP) or oral route. The IP route produces hypertension the fastest, but as described previously the abdominal adhesions as well as a pronounced ascites made this procedure undesirable. Rats were given cadmium acetate IP at a dose of either 2 or 4 mg/kg once a week and their blood pressures measured every 3 or 4 weeks. This was initially done on the Sprague-Dawley rats. After this experience the IP route of administration was abandoned in favor of the oral route.

Three strains of rats were used to determine if there would be any difference in their sensitivity to the effects of cadmium. Also, since this was to be a long term investigation resistance to respiratory infections was also desirable. After four months of drinking water containing 100 ppm of $CdCl_2$ the blood pressures of all animals were measured. The rats of the Long-Evans hooded strain showed the greatest increase in blood pressure. This strain is also known to have a high resistance to disease and was therefore chosen as the animal with which to pursue the bulk of the investigation. The hooded rats

were then allowed to breed so enough animals would be available.

Throughout the breeding the females continued to receive the 100 ppm $CdCl_2$ in their water. There was always a greater mortality in the young born to these rats as compared to controls. These infant deaths occurred at birth and during nursing from the cadmium exposed females.

The blood pressure of the very young could not be determined because their hind feet were too small for the transducer. When their weight was about 150 grams the blood pressures of the young could be monitored. It was then noted that the blood pressures of the young born to mothers who had access to cadmium from conception to birth were higher than the blood pressures of those animals whose mothers did not receive cadmium in their water until the young were born. Table I.

Conflicting reports in the literature make it difficult to state with certainty that cadmium does or does not cross the placenta. Teratogenicity studies make use of cadmium injections at a well-defined time during gestation. Most of these studies used a single large dose (0.5-4.5 mg/kg) given either IV or subcutaneously. Low doses are reported to be stopped by the placental barrier while larger amounts will destroy the fetal placenta and allow the cadmium to cross into the fetus (Friberg et al., 1971). It would appear on the basis of the

TABLE I

BLOOD PRESSURES OF RATS EXPOSED TO CADMIUM PRE AND POST NATALLY

Treatment	Blood Pressure
All Control (n = 25)	129.4 ± 2.7
All Exposed (n = 35)	178.3 ± 1.9[a]
Exposed to Cadmium after Birth (n = 20)	169.0 ± 4.20
Exposed to Cadmium before Birth (n = 15)	190.2 ± 3.98[b]

Values shown are the average ± S.E.M.

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[a] $P < 0.01$

[b] $P < 0.05$

above finding that cadmium is either directly or indirectly exerting an effect across the placenta.

Since the blood pressures of the animals exposed to cadmium in utero are higher than those of animals exposed postnatally, the conclusion would have to be drawn that something was happening before birth. Exposure to cadmium was the only variable which differed between the two groups. The values of the blood pressures are presented in Table I.

It could well be, however, that as cadmium acts to produce hypertension it is getting a "head start" when it is available in utero. Most of the cadmium exposed animals became hypertensive when compared to the controls, but the animals exposed before birth became more severely hypertensive.

B. Effects of Chronic Exposure to Cadmium on Aortic Vascular Smooth Muscle

When aortic strips from control and cadmium hypertensive animals were exposed to several agonists a number of striking differences were seen.

1. Norepinephrine

The aortic strips from cadmium hypertensive animals contracted less than the controls over the entire concentration range of norepinephrine. This is illustrated by Figure 6. These observations are entirely consistent with

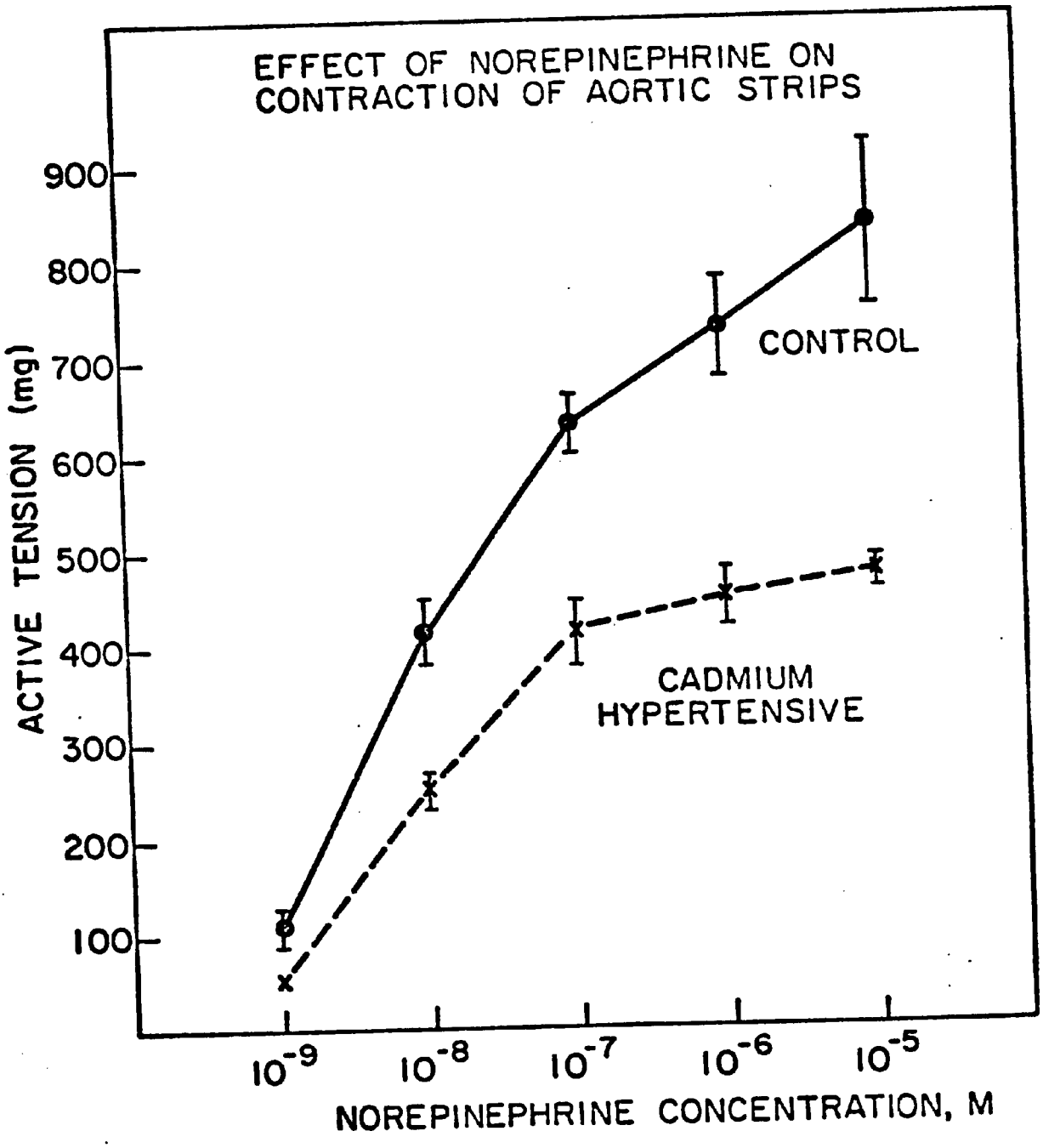
EFFECT OF NOREPINEPHRINE ON
CONTRACTION OF AORTIC STRIPS
ACTIVE TENSION (mg)
900
800
700
600
500
400
300
200
100
CONTROL
CADMIUM
HYPERTENSIVE
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
NOREPINEPHRINE CONCENTRATION, M

Figure 6.

the results obtained by other investigators when hypertensive vascular smooth muscle is exposed to norepinephrine (Bandick and Sparks, 1970; Shibata et al., 1973). It does not appear to make any difference how the hypertension is produced.

2. Serotonin (5-OH-Tryptamine)

There was also a decreased responsiveness observed in the hypertensive as compared to normotensive strips when both strips were exposed to serotonin. These differences can be seen in Figure 7. This response was also noted by Spector et al. (1969) in aortic strips from spontaneously hypertensive rats.

3. Potassium Chloride

The same decreased responsiveness was also noted in the cadmium hypertensive strips when several depolarizing concentrations of KCl were added to the bath. These results are shown in Figure 8. Similar results were reported by Spector and also Field et al. (1972) who saw this same response in aortic strips from both renal and spontaneously hypertensive rats.

The diminished responsiveness seen in the aortic strips from cadmium hypertensive animals to three different agonists can only suggest that these tissues are impaired in their ability to contract. This is a puzzling issue to resolve and to this end the length-tension

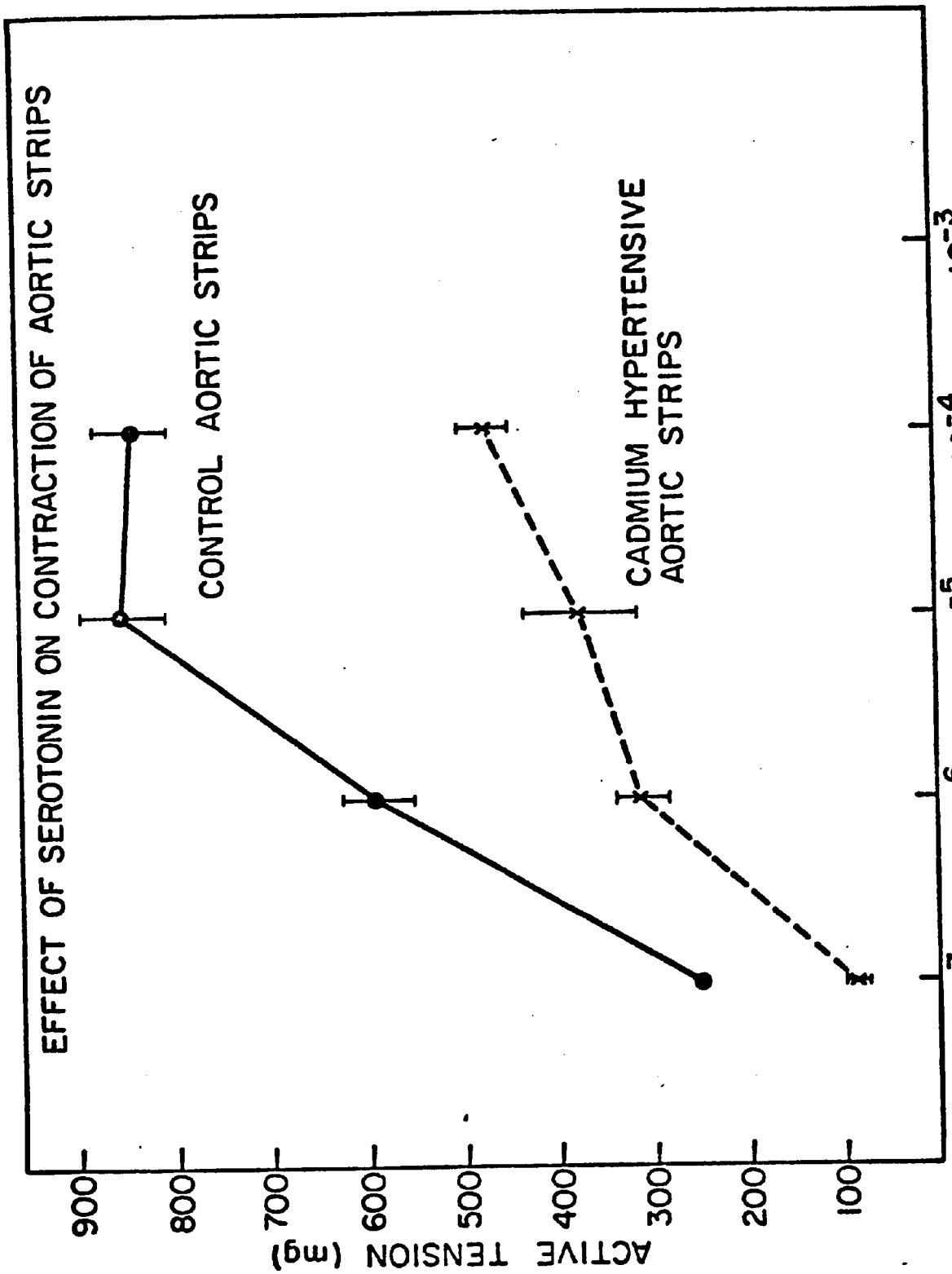
EFFECT OF SEROTONIN ON CONTRACTION OF AORTIC STRIPS
CONTROL AORTIC STRIPS
CADMIUM HYPERTENSIVE AORTIC STRIPS
ACTIVE TENSION (mg)
900
800
700
600
500
400
300
200
100
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
SEROTONIN CONCENTRATION, M

Figure 7.

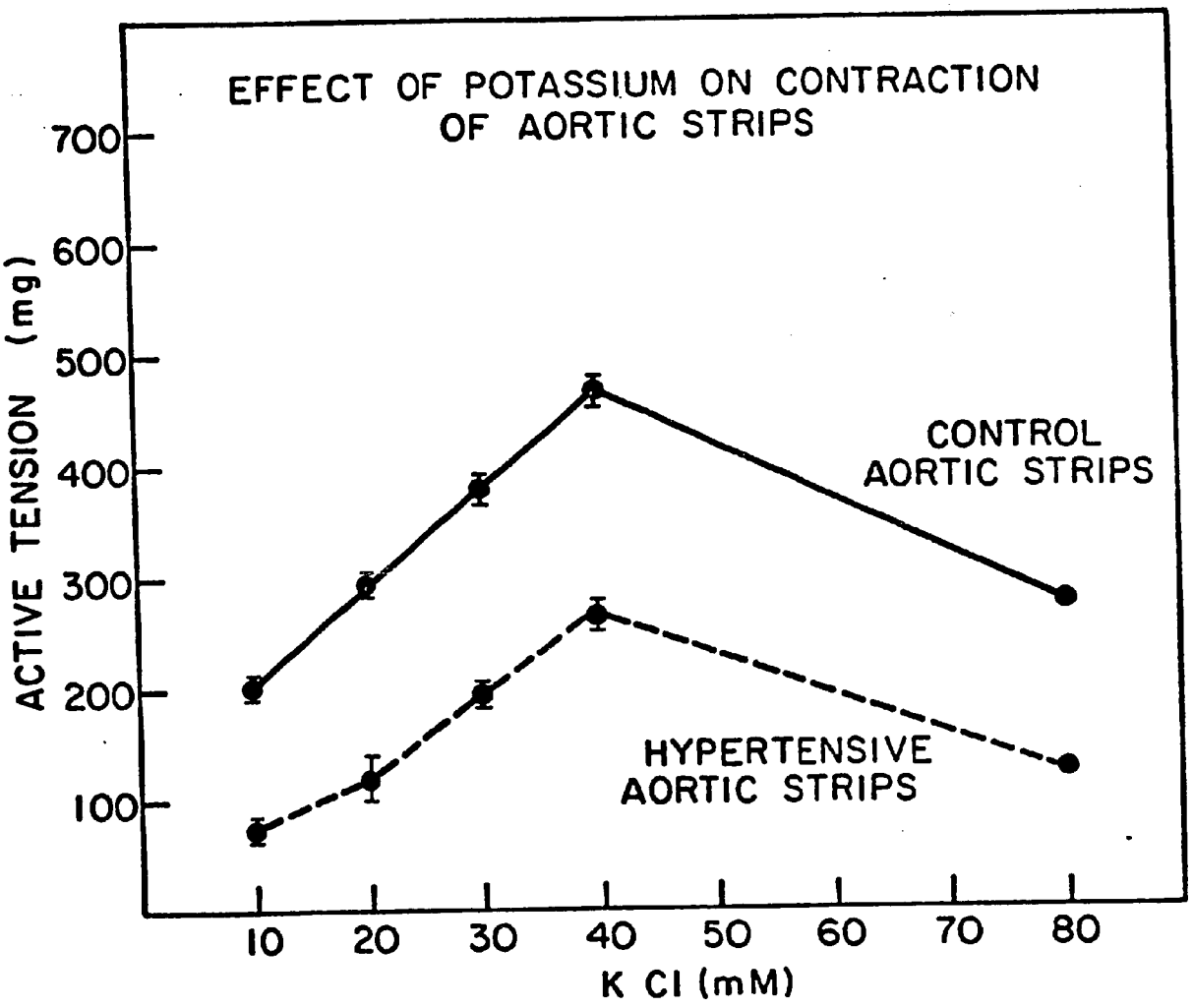
EFFECT OF POTASSIUM ON CONTRACTION
OF AORTIC STRIPS
ACTIVE TENSION (mg)
700
600
500
400
300
200
100
CONTROL
AORTIC STRIPS
HYPERTENSIVE
AORTIC STRIPS
10
20
30
40
50
60
70
80
K Cl (mM)

Figure 8.

studies were undertaken.

C. Length-Tension Studies

The contractile force developed by smooth muscle is a function of its initial length or resting tension. If the muscle is under an increased resting tension more of the contractile proteins are "in use" and would not be available to respond to an additional stimuli (Shibata et al., 1973). This would mean that because of increased stiffness, the hypertensive strip will not have the same initial length as a control strip at any given applied resting tension. Therefore the two strips being compared would always be at different lengths. If the hypertensive strip is in fact stiffer then more resting tension would have to be applied before it would respond maximally as compared to the control strip. This can be studied by varying the amount of resting tension on the aortic strips and determining their response to a single concentration of norepinephrine. If the aortic strips from the hypertensive animals were stiffer than controls, then as the resting tension in the muscle bath is increased one would expect to see a deterioration of the response to norepinephrine in the control strips before it appeared in the hypertensive strips.

When Figure 9 is studied it is apparent that such is not the case. The response of the hypertensive aortic strips exactly parallels that of the control strips through the entire range of resting tensions (1-4 gm). The optimum resting tension for maximum force development was also the same for both cadmium hypertensive and control strips.

D. Response to Cadmium

When cadmium (10^{-4}M) was added to the bathing medium it produced a small contraction ($\bar{X}$ = 123.2 mg; N =14) in the control aortic strips and no response in the cadmium hypertensive strips. Lower concentrations of cadmium (10^{-5}M) had no effect. It is tempting to postulate that the reason the cadmium hypertensive strips do not respond to the addition of cadmium is that they already have responded *in vivo*. This possibility will be further explored in the discussion section. The small contractile response seen in the normotensive strip after addition of cadmium to the bathing medium was abolished in a calcium free medium. This gives some indication that cadmium exerts its effects via a calcium dependent mechanism.

After the cadmium was washed from the bath the control strips would often not return to the original baseline. This occurred despite multiple changes of the bathing medium. Even if this post-cadmium induced contraction did diminish it never returned to the previous

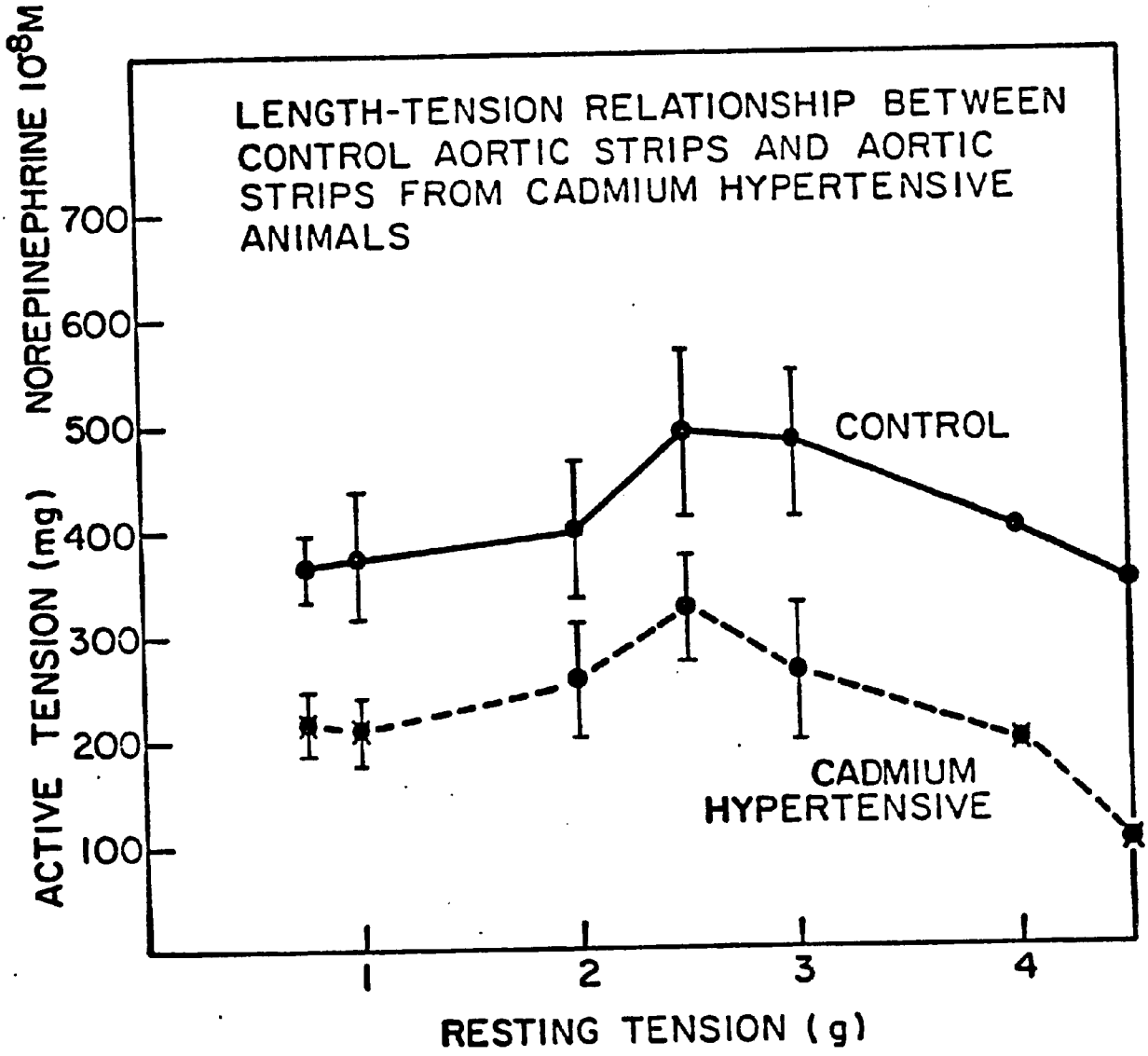
LENGTH-TENSION RELATIONSHIP BETWEEN CONTROL AORTIC STRIPS AND AORTIC STRIPS FROM CADMIUM HYPERTENSIVE ANIMALS
ACTIVE TENSION (mg) NOREPINEPHRINE 10^{-8}M
700
600
500
400
300
200
100
CONTROL
CADMIUM HYPERTENSIVE
1
2
3
4
RESTING TENSION (g)

Figure 9.

baseline. Even a strong relaxing agent such as sodium nitrite ($NaNO_2$ - 100 ug/ml) would only temporarily reverse this contraction. This can be seen in Figures 10 and 11. It was almost as if the cadmium caused the baseline to be reset at a higher level. As noted above, this observation was never made on the vascular smooth muscle from a cadmium hypertensive animal.

E. Effects of $Na_2CaEDTA$

Cadmium hypertension has been reversed, *in vivo*, by the use of chelating agents which presumably remove cadmium from its sites of action (Schroeder and Buckman, 1967). Cadmium ions injected intravenously are also known to be acute pressor agents. The chelating agent $Na_2ZnCDTA$ (cyclohexane-1,2-diamine-NNN'N'-tetraacetate) has also been shown to prevent this acute effect (Schroeder *et al.*, 1970). In light of this type of information it was decided to investigate the responses of aortic strips from cadmium hypertensive animals and controls to the chelating agent $Na_2CaEDTA$ (disodium-calcium-ethylene-diamine tetraacetic acid).

When $Na_2CaEDTA$ ($10^{-3}M$) is added to the bathing medium before cadmium the slight contraction usually seen in the control aortic strips is prevented. If the cadmium contraction is allowed to occur and the $Na_2CaEDTA$ ($10^{-3}M$) is added to the bath after several rinses the original

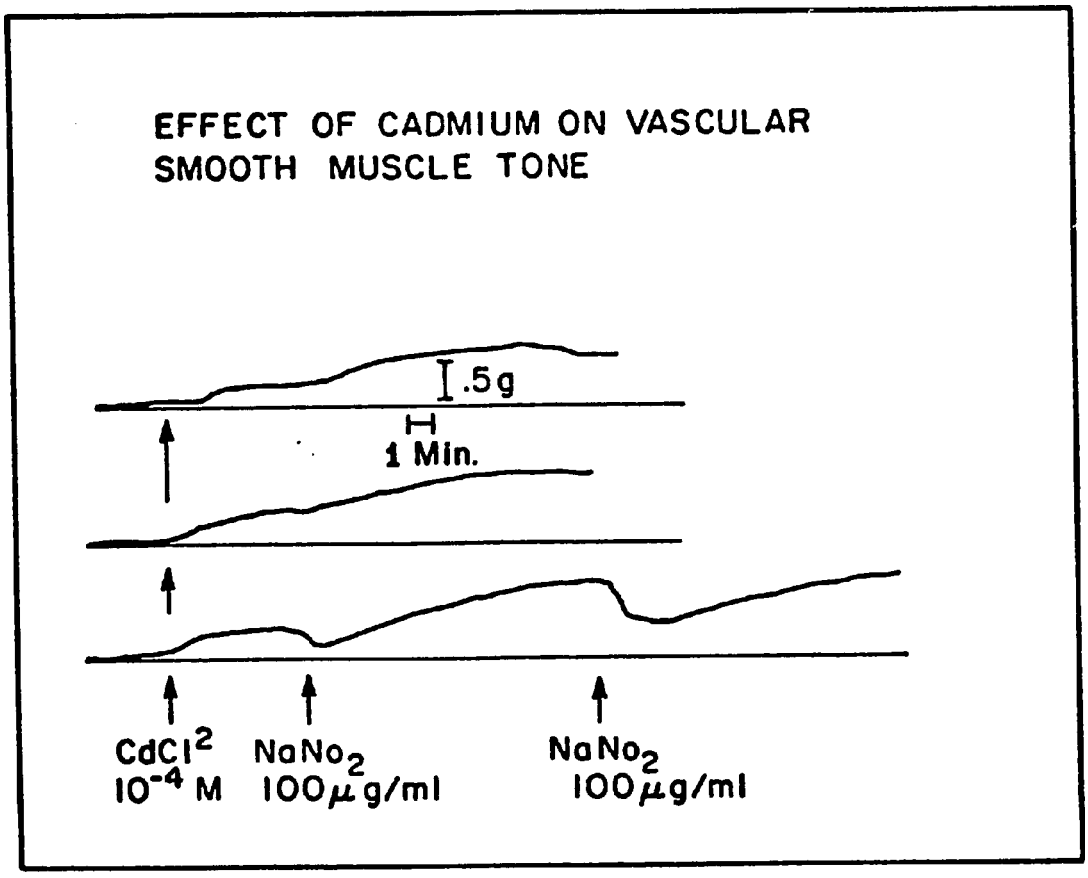

Figure 10. Some typical responses of control aortic strips after exposure to cadmium.

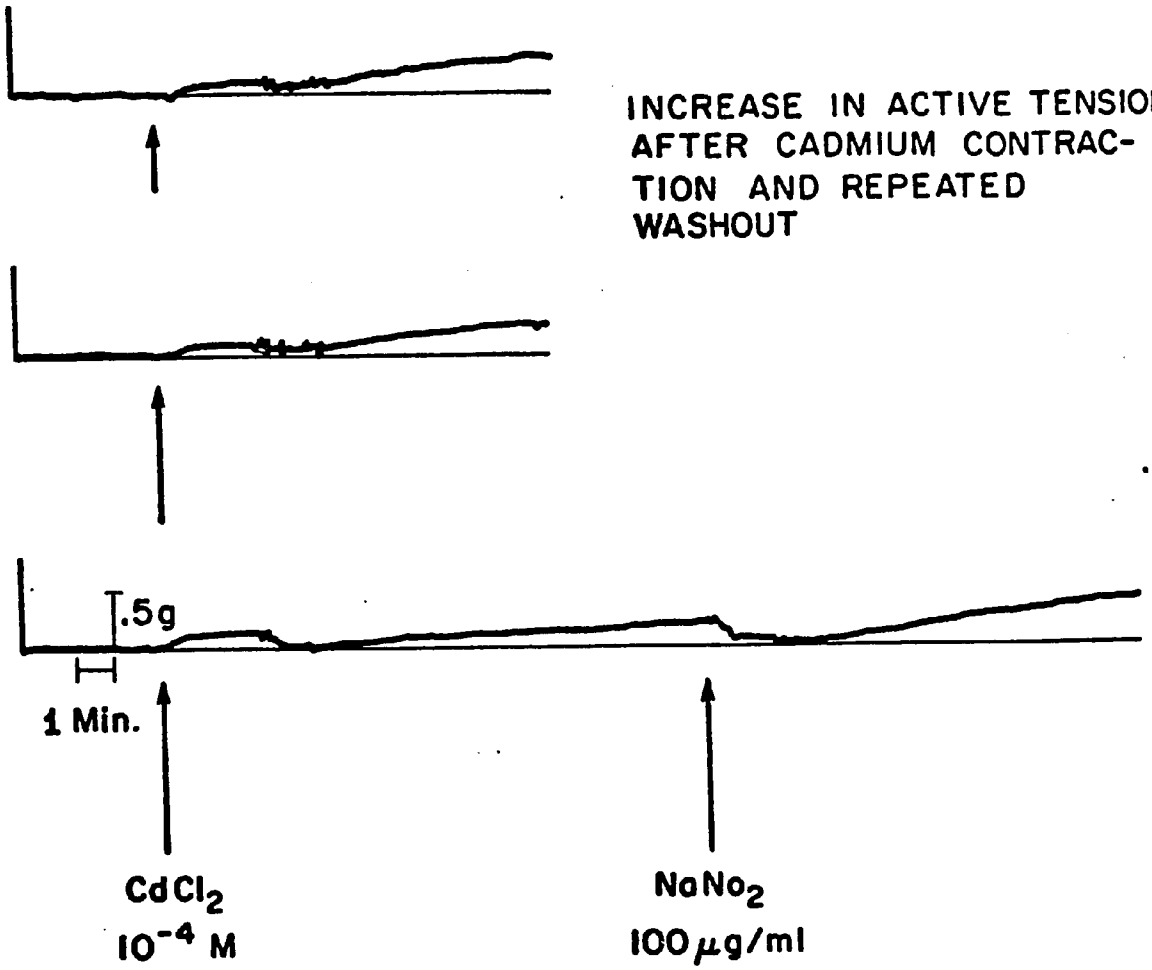

Figure 11. This is a further illustration of the difficulty in reversing a cadmium induced contraction. All responses shown are in control aortic strips.

baseline is promptly restored. It will be recalled from Figure 11 that there was often a problem restoring the original baseline after exposing a control aortic strip to 10^{-4}M Cd^{+2}. Even after many rinses of the bath the strip was still contracting or at least maintaining an elevated baseline. This lingering effect would have to be attributed to the cadmium ion since it is so quickly reversed by the $Na_2CaEDTA$. If the $Na_2CaEDTA$ (10^{-3}M) is added to the muscle bath when both strips are being maintained at resting tension there is no response in the control aortic strips. Cadmium hypertensive aortic strips respond by a decrease in the baseline or resting tension. This decrease in passive tone averaged 125 mg (N = 6) which is almost equal to the amount which cadmium ion caused the control aortic strips to contract (123.2 mg). These two effects are shown in Figure 12.

Since the cadmium hypertensive strips can be made to relax below the imposed resting tension it is likely that the cadmium has produced this effect *in vitro* at least and probably *in vivo* also. To see how much of the difference between the cadmium hypertensive and control aortic strips was due to this mechanism, several of the dose response curves were redrawn. The average decrease in resting tension (125 mg) produced by the $Na_2CaEDTA$ in the hypertensive strips was added to each point on the norepinephrine curve. It will be noted from Figure 13

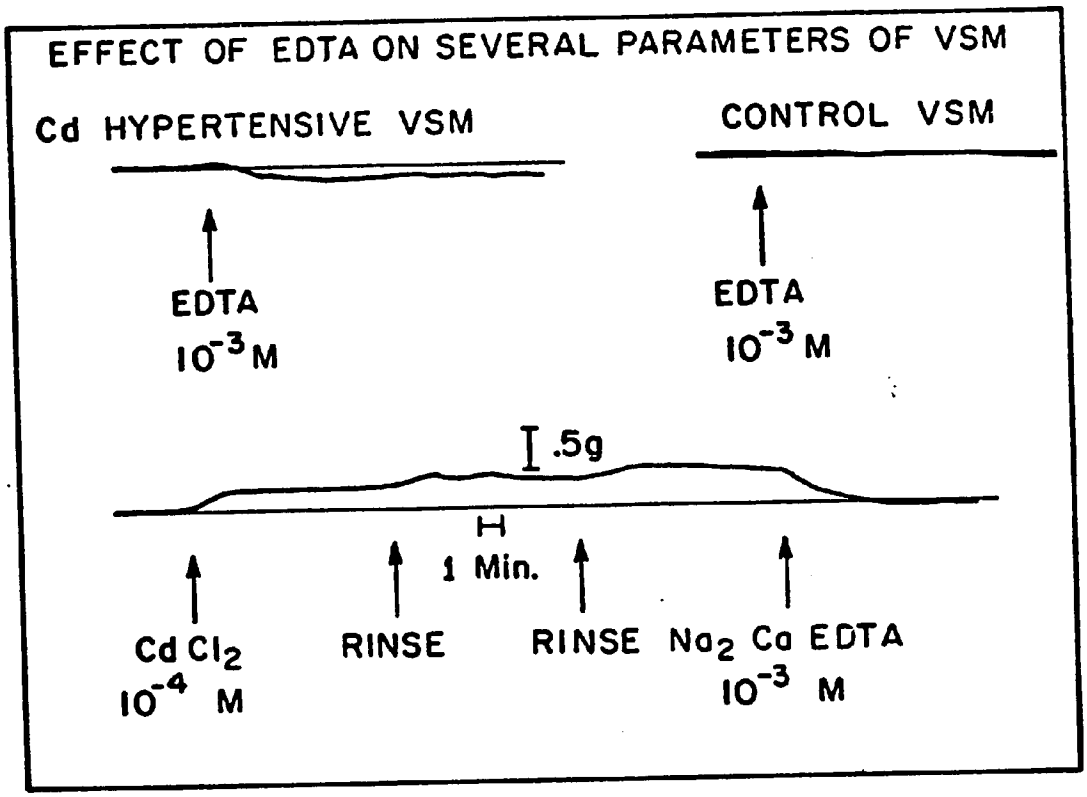

Figure 12. This figure illustrates some of the effects of $Na_2CaEDTA$ on aortic vascular smooth muscle (VSM). The upper left tracing shows the decrease in passive tension in the hypertensive strips as compared to the lack of response in the control strip on the upper right. The bottom tracing shows the immediate reversal of a cadmium contraction by EDTA.

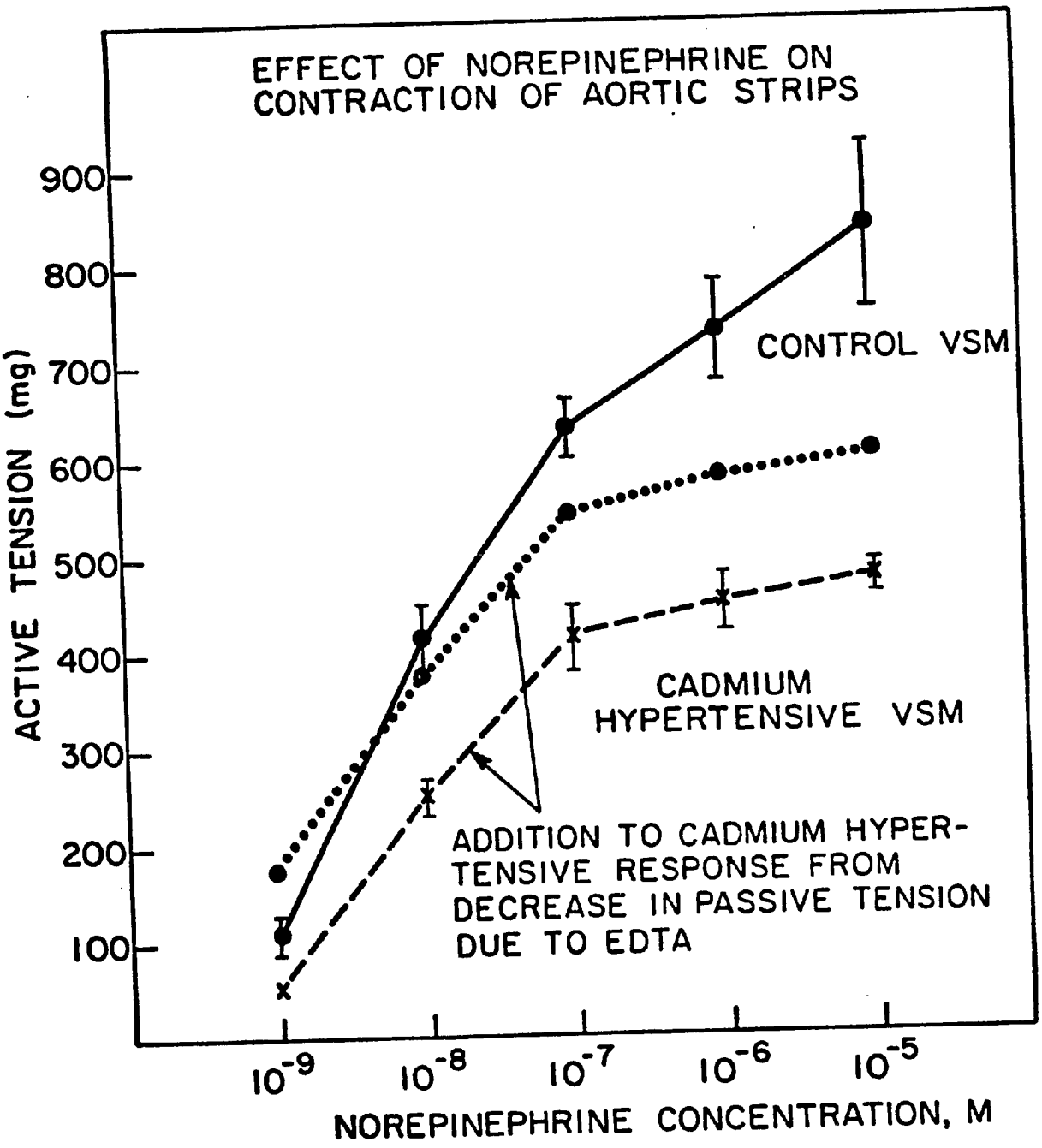

Figure 13. The dotted line in this figure represents the addition of 125 mg to the responses of the hypertensive strips from Figure 6. This was done in an attempt to account for some of the observed differences between the strips in their differential response to EDTA.

that while the corrected curve is close to the control at very low norepinephrine concentrations there is still considerable divergence between the two as the concentration increases. This manipulation may partially account for some of the differences between the two tissues but by no means can all the differences be explained.

This same procedure was carried out on the dose-response curves for K^+ and serotonin. In both of these curves the 125 mg addition moved the experimental closer to the control but could not completely account for the differences between the two tissues (Figures 14 and 15). It is not possible to make the 125 mg addition to each point on the length-tension curves (Figure 9) since the $Na_2CaEDTA$ relaxation was only done at a resting tension of 1.0 g. When the addition is made to this one point almost all of the difference between the two aortic strips disappears. This still does not mean that the action on vascular muscle by cadmium can be explained solely by this observation.

F. Acute Effects of Cadmium Ion

Ionic cadmium has been shown to be an acute pressor agent when administered either intraperitoneally or intravenously. It was therefore decided to study in more detail the responses of aortic strips to the acute effects of cadmium ions.

When vascular smooth muscle responds to norepinephrine each contraction can be divided into two distinct

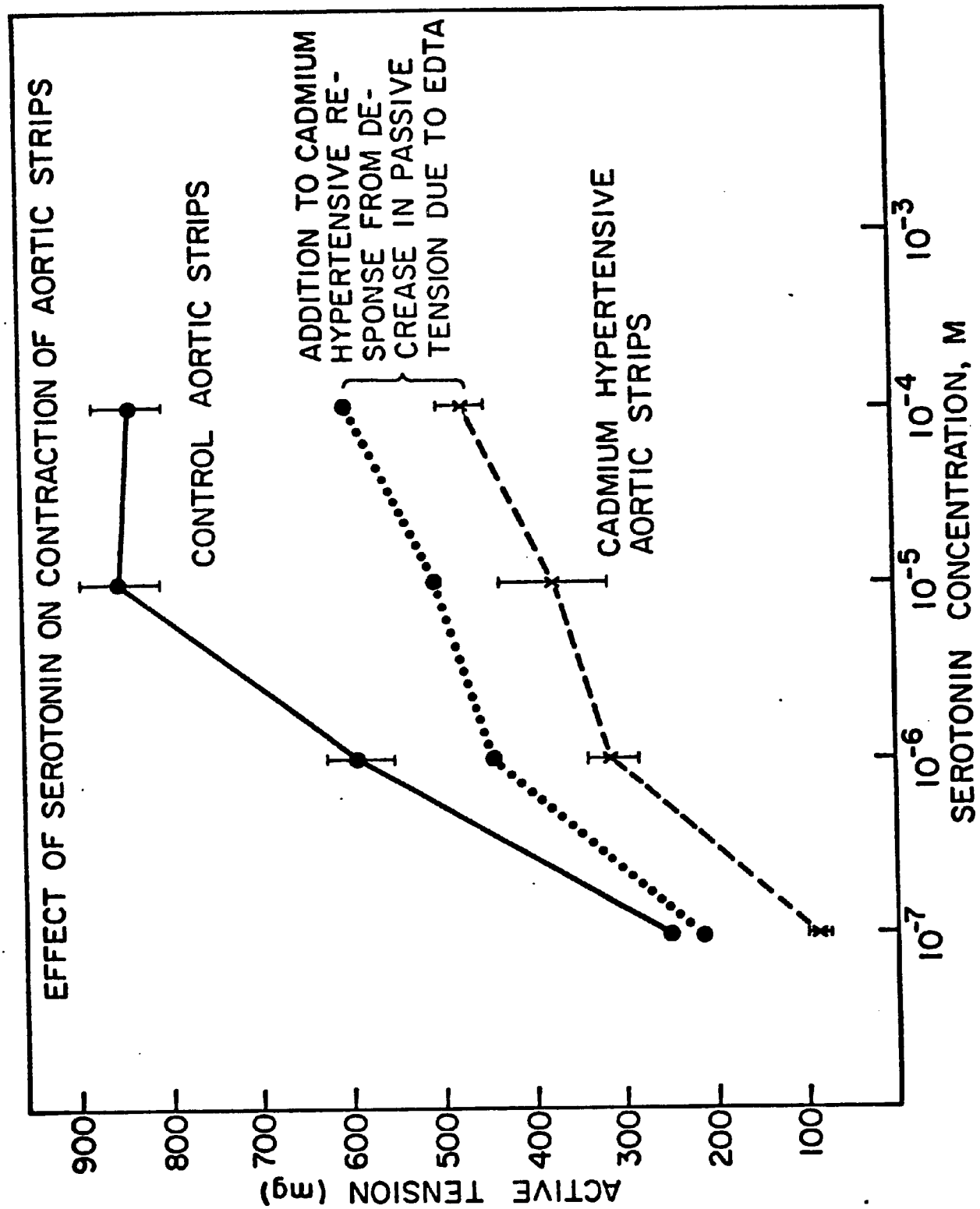

Figure 14. The dotted line in this figure represents the addition of 125 mg to the responses of the hypertensive strips from Figure 7. This was done in an attempt to account for some of the observed differences between the strips in their differential response to EDTA.

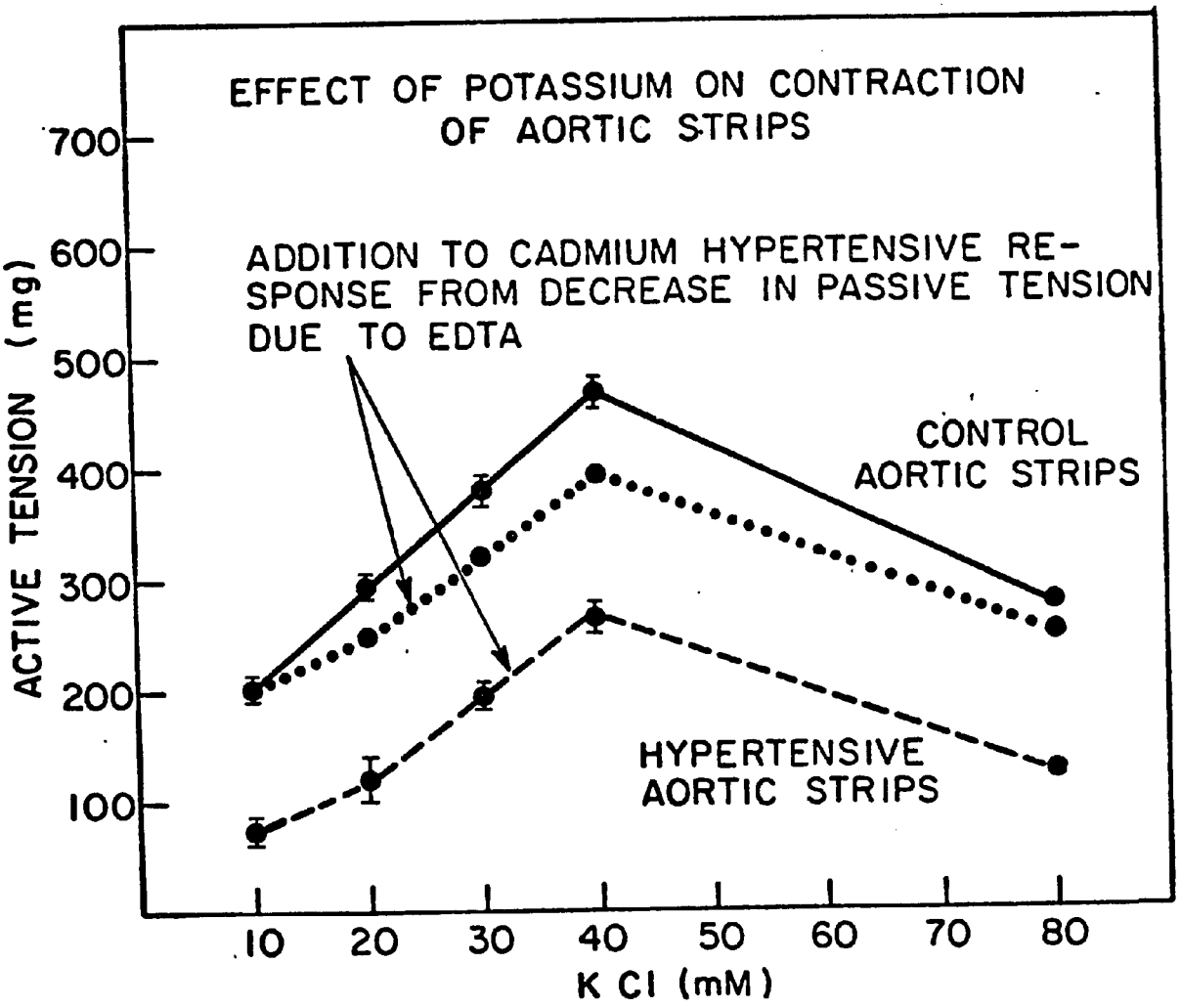

Figure 15. The dotted line in this figure represents the addition of 125 mg to the responses of the hypertensive strips from Figure 8. This was done in an attempt to account for some of the observed differences between the strips in their differential response to EDTA.

components, a fast component which is the result of calcium ions being released from an intracellular pool and initiating the contraction, and a slow component which is due to an influx of extracellular calcium ions (Figure 16) (Godfraind and Kaba, 1972; Steinsland et al., 1973). The slow component is not seen when vascular smooth muscle is stimulated with norepinephrine in a calcium-free medium. This observation was verified in the course of this investigation. Norepinephrine was added to the bathing medium (10^{-5}-10^{-7} M) in the absence of calcium and in 14 observations failed to produce the slow component of the contraction. In this way extracellular calcium is shown to be responsible for the slow component. These concepts will be further reviewed in the discussion section.

If 10^{-4} M cadmium is added to the bathing medium prior to norepinephrine (10^{-5} to 10^{-7} M) only the fast component of the contraction is produced by the norepinephrine. The slow component can then be elicited by the addition of 10^{-3} M $Na_2CaEDTA$ as shown in Figure 16. The $Na_2CaEDTA$ has no effect at all when it is added to the bath after a norepinephrine contraction. In 18 observations there was no evidence that $Na_2CaEDTA$ had any effect when added to the medium after a norepinephrine contraction (Figure 16). If the $Na_2CaEDTA$ is added to the bath before cadmium ion and then the tissues are exposed to

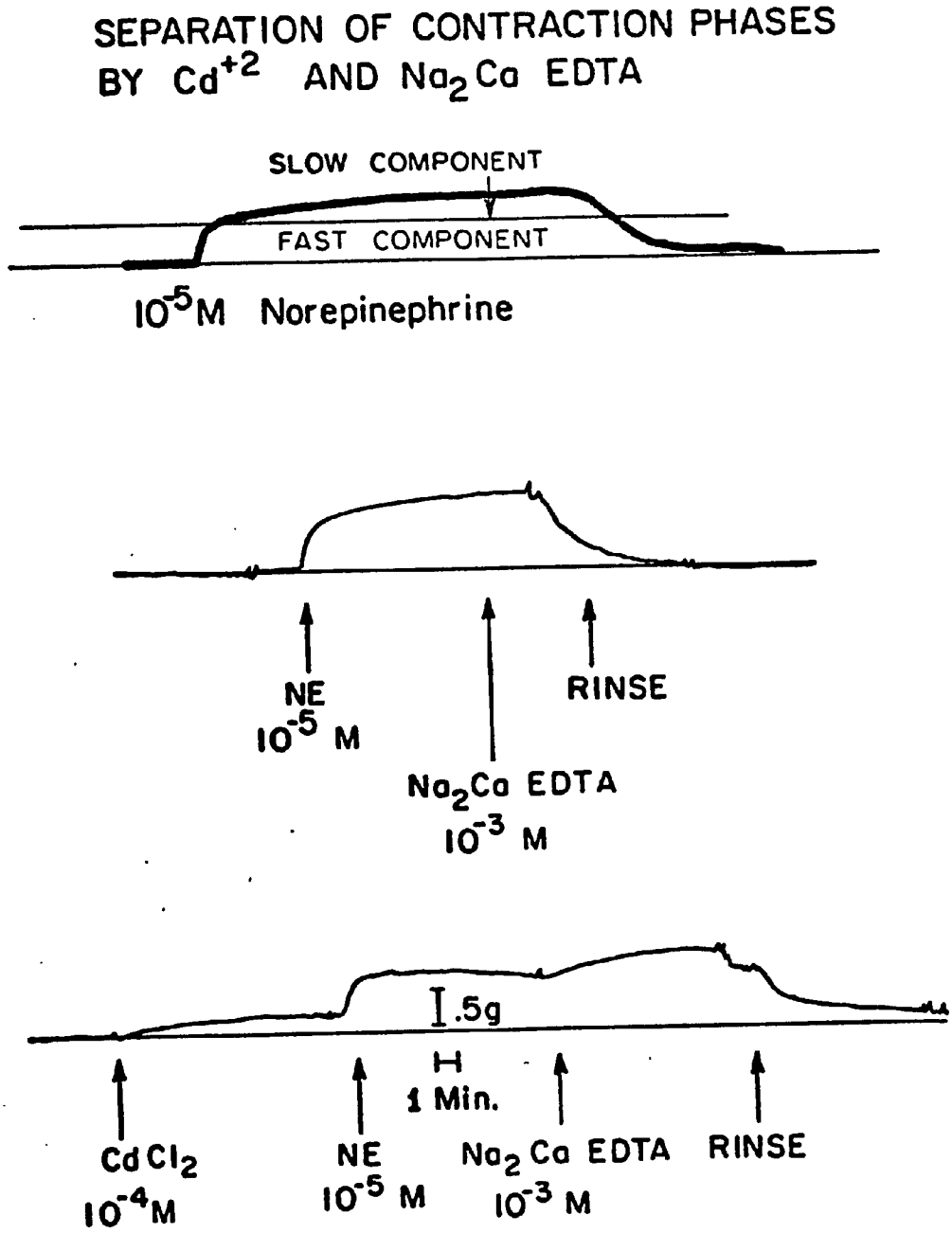

Figure 16. The top tracing shows how each contraction can be separated into two components. The bottom tracing illustrates how cadmium will block the slow component and how EDTA will reverse the blockade of the slow component.

norepinephrine there is a normal contraction. It would appear that whatever action cadmium may have on vascular smooth muscle, is prevented and/or reversed by the addition of a chelating agent such as $Na_2CaEDTA$. This effect has only been noted on an acute basis and is seen in both control and hypertensive vascular smooth muscle even though the cadmium produced no initial response in the hypertensive strip.

Previous studies of contractions of aortic strips from chronically exposed animals were then re-examined to determine if there was a difference between these and controls in the percent of the total response which was due to the slow component. Each contraction can quite easily be divided into the two components and when this was done there was no difference between cadmium exposed and control aortic strips in the proportions that the slow or fast component contributed to the entire contraction. This was true in spite of the fact that the entire contraction of the cadmium hypertensive aortic strips was always less than controls. It would appear, on the basis of these observations, that the chronic effects of cadmium exposure on vascular smooth muscle are not confined to one phase of the contractile response.

G. Effects of Cadmium under Anaerobic Conditions

To study further the acute effects of cadmium ion on vascular smooth muscle, several experiments were performed under anaerobic conditions. This is very easily done by bubbling nitrogen through the bathing medium instead of the normal gas mixture of 95% O_2 and 5% CO_2. Ten to twenty minutes of vigorous bubbling is sufficient to remove any oxygen and render the tissue anaerobic (Detar and Bohr, 1973). If the cadmium induced contraction in unexposed aortic strips is working through an energy dependent process there should be no response in the absence of oxygen. This procedure was done 8 times with the nitrogen bubbling from 10-60 minutes. Cadmium ion (10^{-4} M) was then added to the muscle bath. There was no instance where cadmium ion produced any response under these conditions. There was also a diminished response to norepinephrine under these anerobic conditions.

H. Effects of Lanthanum on Vascular Smooth Muscle

In a further effort to characterize the mechanism of action of cadmium on vascular smooth muscle a series of experiments was performed using the unique properties of lanthanum. There are a number of substances which are known to have a higher affinity for calcium binding sites (extracellular and/or intracellular) than calcium itself. Lanthanum (La^{+3}) a trivalent cation is such a

substance and has been used extensively to study calcium dynamics in vascular smooth muscle (vanBreeman et al., 1973). Physiological solutions to which La^{+3} is added have to be free of carbonate and phosphate ions to avoid precipitation of inorganic La^{+3} salts.

When La^{+3} (1.0 mM) is added to the bathing medium immediately prior to norepinephrine there is a complete block of the slow component and a depressed fast component. These effects can not be reversed with $Na_2CaEDTA$ or washed away with repeated changes of the bathing medium. The La^{+3} has displaced the Ca^{+2} and prevented the vascular smooth muscle from responding (Figure 17). Lower concentrations of La^{+3} (10^{-4} M) will block predominantly the slow component of a norepinephrine contraction. This block is very resistant to the effects of $Na_2CaEDTA$ and can be only partially reversed by concentrations of 10^{-2} M. If La^{+3} (10^{-4} M) is added to the bath before cadmium (10^{-4} M) there is no contraction. The slow component of the norepinephrine contraction is also prevented. As in the case above there is no recovery when 10^{-3} M, $Na_2CaEDTA$ is added to the medium. Higher concentrations (10^{-2} M) of $Na_2CaEDTA$ will show a slight unmasking but only after several minutes of exposure (Figure 18). From these data the conclusion would have to be made that La^{+3} interferes with the action of cadmium by binding more strongly to whatever sites cadmium would usually

EFFECT OF LANTHANUM ON CONTRACTILE RESPONSE
OF VASCULAR SMOOTH MUSCLE

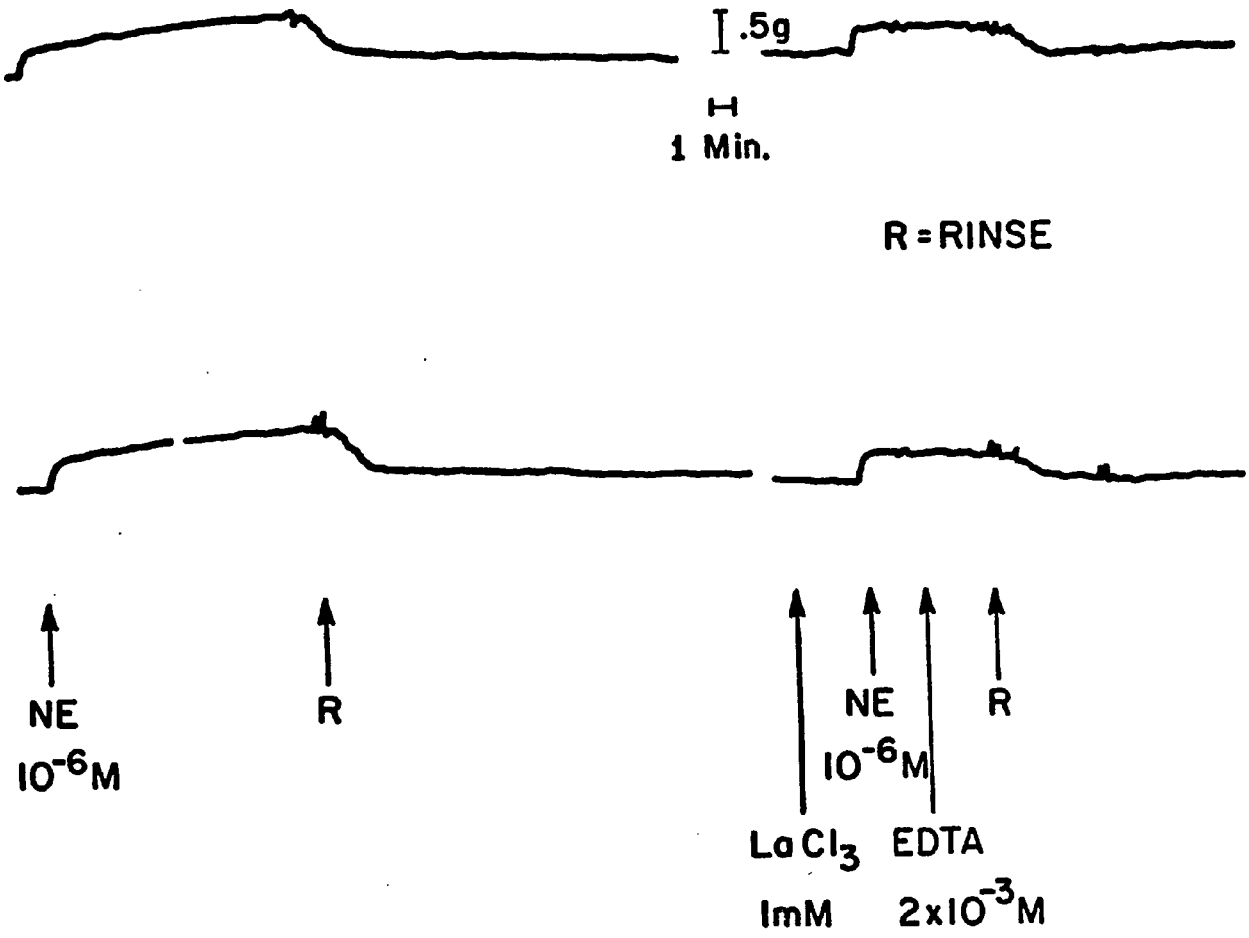

Figure 17. Lanthanum is also able to block the slow component of a NE contraction as shown by the two right tracings in this figure. EDTA is not able to reverse the blockade produced by lanthanum.

INTERACTIONS BETWEEN CADMIUM AND LANTHANUM ON VASCULAR SMOOTH MUSCLE

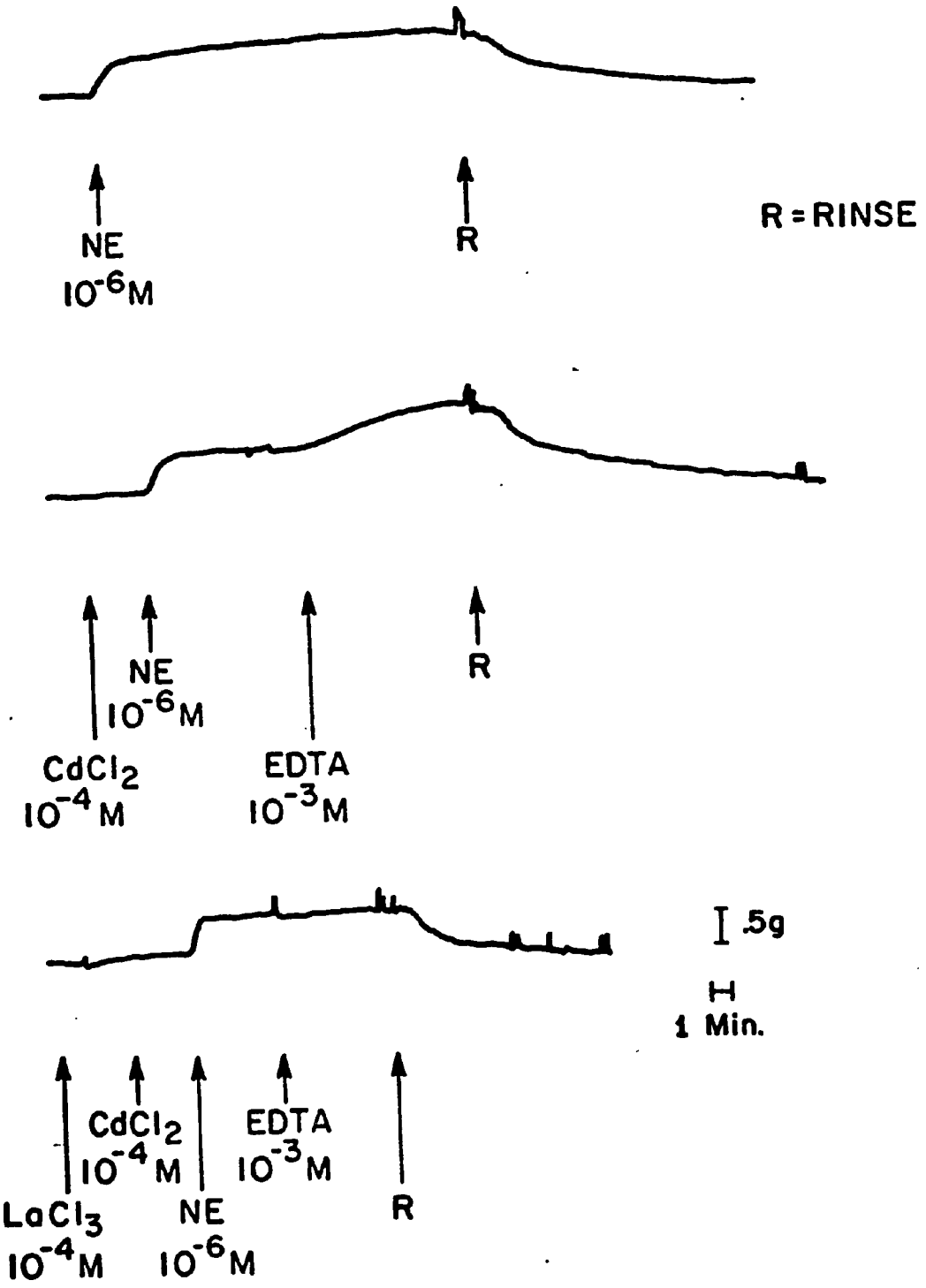

Figure 18. When lanthanum preceeds cadmium the slow component is blocked and EDTA will not reverse the blockade and restore the slow component.

occupy. This information will be fully integrated into an overall explanation in the discussion section.

I. Tissue Cadmium Levels

Since there was a large number of animals exposed for up to two years to cadmium an excellent opportunity was presented to measure tissue levels of this element. Over an extended time, cadmium is known to concentrate mostly in the kidney and secondarily in the liver. All other organs concentrate cadmium significantly less (Friberg et al., 1971). These two organs were therefore assayed for cadmium and because the focus of this investigation was on vascular smooth muscle, the thoracic aorta was also assayed for cadmium. The analytical method used was not sensitive enough to detect any cadmium in a single throacic aorta (average weight - 0.015 g) so it was necessary to pool the aortas from 6-8 animals to establish a tissue cadmium concentration in the aorta.

Needless to say, there was a great and significant difference in all tissue cadmium levels between exposed and control animals. The most interesting findings were seen when the data from the exposed group of animals were examined more closely. Table II illustrates this point. There appears initially a significant difference between male and female animals with respect to the amounts of hepatic and renal cadmium. It must be remembered though,

TABLE II

ORGAN WEIGHTS AND CADMIUM LEVELS OF EXPOSED AND CONTROL RATS

Sex	Tissue	Organ weight (g)	Cadmium (ug/g)
F	Liver	8.26 ± 0.4	33.2 ± 3.7
M	Liver	15.32 ± 0.9	13.9 ± 1.1[a]
F	Kidney	2.17 ± 0.6	50.8 ± 5.0
M	Kidney	3.21 ± 0.2	32.3 ± 3.2[b]
	Aorta (pooled)		0.87
	Liver (control)		0.32
	Kidney (control)		0.31

[a] $p < .0001$

[b] $p < .001$

that there is also a significant difference between the weights of liver and kidney in the males and females. After computing the total hepatic and renal cadmium from assay results and organ weights the significant difference between the sexes disappears. Table III shows the differences between males and females and organ cadmium concentrations. However this apparent similarity between the sexes in the computed partial body burden is deceptive. When the computed partial body burden is divided by the body weight of each animal there appears a very significant difference between the sexes. It will also be noted that on an overall comparison of blood pressures the female rats averaged higher blood pressures than the males. The differences are not significant.

Female Average Blood Pressure = 185.3

Male Average Blood Pressure = 172.6

J. Half-life

Approximately six months before the termination of the research ten animals which had been receiving cadmium in their drinking water were removed from this exposure. This was done to get an idea of how long a body burden of cadmium would remain. The half-life of cadmium is known to be very long and varies from species to species. The half-life in mice for example, is thought to be about 200 days while for monkeys it is around 2 years. The newest estimates in man for total body half-life are

TABLE III

TOTAL LIVER AND KIDNEY CADMIUM LEVELS AND PARTIAL COMPUTED BODY BURDENS

Tissue	Sex	Total cadmium (ug)
Liver	F	262.9 ± 26.8
Liver	M	216.8 ± 22.0
Kidney	F	109.1 ± 10.6
Kidney	M	104.2 ± 11.8
Partial body burden (liver & kidney)	F	371.9 ± 34.8
Partial body burden (liver & kidney)	M	321.0 ± 32.7
		(ug cadium/kg body weight)
Partial body burden computed on basis of body weight	F	1354.8 ± 138.0
	M	638.3 ± 52.1[a]

[a] $p < .0001$

between 10-30 years. These determinations are difficult at best, since so many variables must be considered. Sex differences, species differences, route of administration, type of compound, previous body burden as well as the mathematical extrapolation are but a few of the problems involved in arriving at an estimate of a biological half-life.

Cadmium is initially stored in the liver and is then transferred to the kidney where it is bound by a specific protein—metallothionein. This is a protein with many unique properties. It has a molecular weight of around 10,000 and in addition contains about 9% sulphur due to a high percentage of cysteine in the protein. Metallothionein has the ability to bind large amounts of cadmium and zinc. In metallothionein isolated from horse kidney, the cadmium content was 5.9% and the zinc content 2.2%. Metallothionein appears to be syntheized in the liver and then transferred to the kidney. It has been found in the kidneys of rats, rabbits, horses and man (Friberg et al., 1971). One could predict on the basis of this information that the liver cadmium would drop by a greater amount than the kidney cadmium subsequent to removal of an animal from exposure.

The observations concur with this prediction as can be seen from Table IV. The amount of decrease is greater in the liver than the kidney.

TABLE IV

DECREASE IN CADMIUM LEVELS AFTER STOPPING CADMIUM EXPOSURE

Source	Cd-exposed (ug)	Removed from Cd (ug)	% Decrease
Total liver	239.82	165.12	31
Total kidney	106.63	82.90	22
Partial body	346.45	248.02	28

Although not enough data are available to completely answer the half-life question, the results obtained in this study are certainly within the range suggested by the literature. These animals were removed from cadmium exposure approximately 180 days prior to cadmium analysis. The partial body burden, as computed by combining total liver and kidney cadmium concentrations, was decreased by 28% as compared to those animal maintained on cadmium until the termination of the research. When the half-life is computed according to the equations: $N = Noe^{-\lambda t}$ and $t_{\frac{1}{2}} = \frac{0.693}{\lambda}$ the resulting figure is 371 days. This agrees quite favorably with a reported half-life of 300 days in rats (Friberg et al., 1971). These calculations treat the clearance of cadmium from the rat as a first order clearance rate from a single compartment.

K. Pathology

There were no differences between cadmium exposed and control rats with regard to the microscopic appearance of the liver.

Various forms of hypertension have been associated with increased arterial wall thickness and an increased wall to lumen ratio (Bandick and Sparks, 1970; Folkow, 1971). Because of these findings the aortic wall thickness was compared in the cadmium exposed and control animals. This was done by projecting the image of the microscopic slides onto a screen and measuring the thickness of the aorta. Each aortic cross section was measured at four positions and these were then averaged. There was no difference in the aortic wall thickness between the two groups. No absolute values can be given since the measurements were only arbitrary units which were a function of the particular (constant) distance between the projector and the screen.

The kidneys were the only organs in which pathologic changes were noted in exposed rats. The lesions, when present, were characterized by various degrees of dilatation of collecting tubules and the presence of a proteinaceous material (eosinophilic) in many of the tubules. There was also some epithelial vacuolization in the convoluted tubules. An attempt was made to grade the above described lesions on a scale of 1+ - 4+ according to their severity. Figures 19 and 20 illustrate the type and degree of damage



Figure 19a. Tubular damage graded 1+ on the basis of eosinophilic material observed in tubules. H & E stain 35X



Figure 19b. Renal damage graded 2+ on the basis of eosinophilic material in tubules along with slight distention of collecting tubules. H & E stain 35X

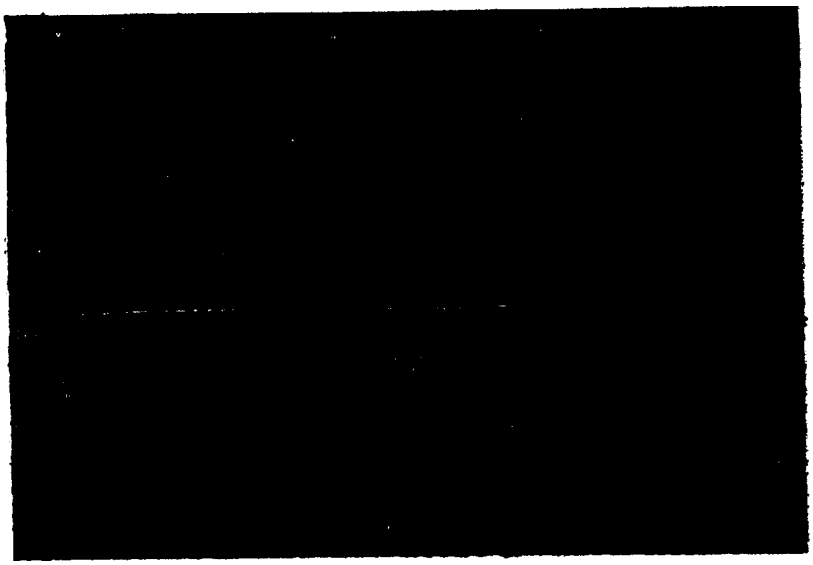

Figure 20a. Renal damage graded 3+. Note large amounts of eosinophilic material filling and dilating tubules and subsequent alteration of normal renal morphology.
H & E stain 35X



Figure 20b. Most severe renal disease observed-graded 4 +. Severe destruction of tubules is noted as well as a marked displacement of normal renal morphology due to dilatation and vacuolization of collecting tubules.
H & E stain 35X

assigned to each graduation (1+ - 4+) of renal lesion. Almost all of the kidneys where tubular damage was evident were also observed to have various amounts of interstitial inflamatory infiltrates.

Renal damage of some degree was observed in 50% of the controls and 53% of the cadmium exposed group. When only the 3+ - 4+ lesions are included the percentages are 7% and 22% respectively. There also appeared to be an increased arteriolar wall to lumen ratio in those kidneys which were graded 3+ - 4+. This increased ratio was therefore seen more frequently in the cadmium exposed animals.

A striking difference in renal lesions was noted when animals were grouped according to sex. The control group showed 43% of females and only 7% of males with some degree of renal damage. In the cadmium exposed group 34% of females and 19% of males had renal lesions. If only the 3+ and 4+ renal lesions are considered, the control rats showed 11% of females and 0% of males while in the cadmium exposed rats, 31% of females and 13% of males showed this degree of renal disease.

Another unexpected finding was noted in the animals which were born to mothers with cadmium in their drinking water. Fully 71% of the exposed group which showed any evidence of renal damage had been first exposed to cadmium <u>in utero</u>. This finding is made more dramatic when it was noted that 92% of all rats exposed before birth

demonstrated some degree of renal disease. It will be remembered that this is the same group which demonstrated an increased incidence and severity of hypertension. Other than this observation there was no correlation between severity of hypertension and presence of renal disease.

DISCUSSION

The task of interpreting the results of this research would be much easier if there were an overall unified understanding of the pathophysiology of hypertension. Despite the large amount of data collected in recent years concerning hypertension the underlying mechanisms are still in doubt.

A. Control of Blood Pressure

Very basically blood pressure is controlled by two factors, the cardiac output, which is the amount of blood pumped by the heart per unit time, and the peripheral vascular resistance which is the relative state of constriction or relaxation of the arterial system into which the blood is pumped. There are of course many factors which can alter either one of these and therefore change the blood pressure. Hypertension results when one or both of these factors are affected in such a way as to cause the blood pressure to be reset at a higher than normal level.

In the usual forms of arterial hypertension the cardiac output is fairly normal while there is increased peripheral resistance. This increased peripheral resistance must include vessels in the kidney because if these

arterioles are not involved the kidney will cause the blood pressure to normalize. In fact renal vessels have been shown to be involved in all forms of chronic hypertension. This does not, of course, mean that they are the only vessels involved.

A great deal is known about how arteries respond in hypertension but there remains a number of confusing paradoxes. Most systemic arterioles are known to have an increased wall thickness and narrowed lumen during chronic hypertension. This increased thickness is due partly to an increased water content as well as a hypertrophy of the smooth muscle cells (Folkow, 1971). The problem remains to explain what caused the hypertrophy and edema of the vessel wall.

The puzzle lies in the fact that hypertrophied smooth muscle cells often have been shown to contract less than those of normal arteries. This has been observed in the arteries from animals whose hypertension was produced in a number of different ways (Shibata et al., 1973). Further confusion arises because the opposite has also been observed-that is vascular smooth muscle from hypertensive animals has also been shown to exhibit a greater responsiveness to the stimulating agents KCl, norepinephrine, and serotonin (Field et al., 1972; Holloway and Bohr, 1973). Trying to resolve differences of this nature is particularly perplexing in light of the fact that these extremes

have been observed by many conscientious investigators.

B. Control of Vascular Smooth Muscle Tone

The basal tone of vascular smooth muscle is controlled by a number of related events. There is always a certain basal level of contraction of the smooth muscle cells. This represents the basal tone which is related to the maintenance of a normal blood pressure. Some of the related factors which act to maintain this tone are as follows: a more or less steady state release of norepinephrine stimulates the right number of smooth muscle cells to maintain this tone. A number of specific ionic pumps maintain concentration gradients of calcium, sodium and potassium within certain narrow limits. Mechanisms to insure that intracellular pools of calcium are maintained in such a way that calcium does not become available to activate more contractile units than are needed. These and other factors act in concert to hold a constant tone but still respond quickly to stressful situations.

It must be pointed out that many arteries are able to maintain a normal tone after complete denervation (Celander and Folkow, 1953). This would indicate that there is a strong myogenic or humoral component which is important in regulating peripheral resistance. Just how this myogenic

tone-regulating mechanism functions is still open to some speculation. Some femoral strips have been shown to exhibit a spontaneous rhythmicity which could be related to a tone setting mechanism (Bandick and Sparks, 1970). Another probable component of the tone setting mechanism is calcium. Because calcium is found in great abundance outside of the cell, membrane pumps must operate continuously to keep the intracellular environment free of excess ionic calcium. In view of the large concentration gradient some inward leakage of calcium would appear possible. This concept is supported by the work of Uchida and Bohr (1969) on the myogenic tone of isolated, perfused resistance vessels. They concluded that myogenic tone was abolished in a calcium free medium. They further noted that pressure (blood pressure) was not a necessary factor for the maintenance of this myogenic tone. To eliminate the possibility that catecholamines were somehow involved, artery preparations from reserpinized rats were studied. These vessels were no different from controls in the amount of myogenic tone demonstrated.

Two possible explanations are offered to explain the mechanism of myogenic tone. The first is that those vessels showing myogenic tone have a decreased ability to trap calcium adequately at the cell membrane. Thus, the membrane is unable to trap enough calcium from the high

extracellular gradient and there is always enough free intracellular calcium to cause some percent of the contractile proteins to develop tension. The second interpretation is that there is simply a greater membrane permability to calcium in those vessels which have a higher myogenic tone. Both concepts have in common the idea that excess intracellular free calcium activates enough contractile protein to produce a myogenic tone.

All of these related factors are then "set" to maintain the normal blood pressure much in the same way as a thermostat is set to hold a certain temperature. Hypertension will result when something interferes with this homeostatic mechanism and causes it to be reset at a higher point.

It is quite easy to understand how, for example, a pheochromocytoma which hypersecretes epinephrine can elevate blood pressure by causing generalized vasoconstriction. On the other hand it is more difficult to grasp the mechanism behind essential hypertension. This is an extremely complicated entity which probably involves interrelationships between heredity, stress, diet, renin-angiotensin, humoral substances as well as other undiscovered factors. With the situation as complicated as this it is difficult to identify one cause as the culprit. Nevertheless it is possible to produce hypertension in animals by performing very simple, yet specific manipulations. This is done in the hope that hypertension

produced in one of these ways will mimic essential hypertension closely enough that inferences can be made as to the mechanism of this disease.

Cadmium has already been mentioned as a possible contributing cause for essential hypertension. It would be naive to suggest that cadmium was the sole causative agent in essential hypertension, but the implications of its involvement can hardly be overlooked.

C. Production of Cadmium Hypertension

There can be no doubt that cadmium alone, whether administered orally, IV or IP, can produce significant hypertension. This was confirmed throughout the course of this investigation.

Hypertension was initially produced by injecting cadmium intraperitoneally. While this caused the animals to become hypertensive the ascites and adhesions which developed were considered to be a drawback. Even though the vehicle was 0.9% NaCl the presence of the cadmium was enough of an irritant to produce these effects. It is rather curious that there is no literature which mentions this effect and yet it was observed by another investigator in this laboratory. These problems were eliminated by using the oral route of administration. The production of hypertension has already been covered and will not be further discussed. One finding which was quite unexpected

was the greater hypertension seen in the young rats born to mothers who had been exposed to cadmium from conception through birth and postnatally. The only way to account for this is that some of the dietary cadmium found its way through the placenta and was then available to the developing fetuses. The cadmium could then possibly interfere with the developing vascular tone setting mechanism and thereby get a "head start" in producing hypertension. Another possible explanation for this finding would be that some exogenous factor from the mother was released after cadmium exposure and crossed the placenta to help induce hypertension.

Cadmium has been studied for its teratogenic effects but it has always been administered in rather large amounts (2-13 mg/kg) on specific days of gestation. The most common anomalies seen after experiments of this type were various skeletal defects and hypoplastic lungs (Chernoff, 1973; Gale, 1973). No gross defects of any kind were seen in any of the many litters born throughout this investigation. When the animals were exposed to only 100 ppm cadmium in their drinking water there was no opportunity for even the lowest dose previously reported as teratogenic to be approached.

The fact that there was a significant difference between the blood pressures of rats exposed before birth as compared to those exposed after birth would seem to indicate that the cadmium was having an effect. It is also important to note that these animals maintained a

higher average blood pressure over the entire observation period than those exposed after birth. This would indicate either they had higher pressures at birth or perhaps an alteration of the arterial vascular smooth muscle before birth which continued to keep their blood pressures ahead of the others. Whether this was some kind of conditioning effect or a direct effect on a blood pressure control point is not known. It is also possible that cadmium exposure *in utero* produces some kind of developmental defect in the vascular smooth muscle.

D. Effects of Several Agonists on Response of Aortic Strips

There have been hundreds of studies done on the responses of aortic strips, or other vascular smooth muscle, to many agonists. Many have observed a difference between hypertensive and control aortic strips under a variety of conditions. Often this difference is seen as a decreased responsiveness to norepinephrine, for example, by hypertensive strips as compared to controls (Spector *et al.*, 1969; Bandick and Sparks, 1970; Field *et al.*, 1972). These as well as other investigators have also observed this same difference between aortic strips when they were exposed to potassium and serotonin.

There is only one report in the literature on vascular reactivity in cadmium hypertensive animals (Thind *et al.*,

1970). This work was done on the aorta of cadmium hypertensive rabbits. These investigators reported a decreased responsiveness to angiotensin but no difference between the aortic strips in response to norepinephrine. This latter observation was explained by stating that in general most hypertensive vessels exhibit an increased responsiveness to norepinephrine. While this may not be an adequate explanation the fact that there is such variation in these kinds of observations makes it at least as good as any other.

The results obtained in this study agree quite favorably with those seen in other forms of experimental hypertension. Cadmium-hypertensive aortic strips exhibited decreased responsiveness to norepinephrine, potassium and serotonin as compared to normotensive strips. A similar type of observation was made by Schroeder et al. (1970) in an in vivo situation. They too concluded that the presence of cadmium in the rat appeared to render vascular reactivity less sensitive to the pressor effects of norepinephrine and angiotensin.

This is a difficult problem to resolve in all types of experimental hypertension because of a feeling that these strips ought to contract more than controls. Since most hypertension is associated with an increased peripheral resistance, this phenomenon, it might be argued, should be translated into an increased responsiveness in vitro. Because this is often not the case other explanations must be considered.

Differences in responsiveness between hypertensive and normotensive strips could be explained if the hypertensive strips have had a greater amount of spontaneous tone or resting tension. The amount of force developed by smooth muscle is a function of its initial length or resting tension. A suggestion has been made by some (Redleaf and Tobian, 1958) that hypertensive vessels have an elevated spontaneous tone. This would mean that in a resting state more of the contractile proteins are contracted and are not available to respond to a stimulus. Therefore a piece of vascular smooth muscle in this condition would not be able to contract as much as one with more available contractile protein. As increasing resting tension is applied to an aortic strip a point will be reached where the elastic limit of the tissue is exceeded and a decrement in response will be seen. If a hypertensive strip is in fact under greater spontaneous tone then this failure should be seen at an applied resting tension which is less than that applied to a normotensive strip. Since the active tension developed in the hypertensive strips was always lower but parallel to the normotensive strips this explanation for the differences between the two tissues does not appear to be valid. These same observations were also made by Shibata (1973) when he compared aortic strips from spontaneously hypertensive rats to normotensive rats.

There is also considerable evidence which shows that arteries from hypertensive animals have an increased water content as well as increased Na, K, Cl, and Ca (Tobian, 1952; Jones et al., 1964). These increases are reflected in greater arterial wall thickness and might also be associated with an increased stiffness of hypertensive arteries. This explanation too is invalidated by the results of the length-tension studies. If the hypertensive aortic strips were in fact stiffer than the normotensive strips there should have been an increase in active tension which occurred after that seen in the control strips. Since this did not occur one would have to conclude that differences in stiffness or stretchability can not be used to explain the diminished responses seen in the hypertensive aortic strips.

Another factor which must be considered is that cadmium hypertensive animals have a higher level of circulating renin than normotensives. There is a report by Perry and Erlanger (1973) that cadmium was able to elevate circulating renin activity in rats. They found that a single injection of cadmium (1.8 uM) was able to raise renin levels in one minute and this elevation persited for one month. Also noticed was the fact that when rats were continuously exposed to cadmium in their water their renin levels were significantly increased after one week and one month, but not after 3 months. It would, therefore, appear that the cadmium hypertension greatly

outlasts any increases in renin levels. Whether the increased renin is necessary for the production of cadmium hypertension is not known.

E. Responses of Aortic Strips to Cadmium Ion

A most interesting observation was made when cadmium (10^{-4}M) was added to the bathing medium. It produced contractions in the normotensive strips and nothing at all in the hypertensive strips. This finding could be explained by assuming that the hypertensive strip has already responded to the cadmium in vivo. While this cadmium-induced contraction can not completely account for the differences seen between strips it does indicate that cadmium has a direct effect on vascular smooth muscle.

The similarities between the cadmium and calcium ions have already been mentioned. Calcium serves as an activator of the contraction process. When calcium is added to the physiological salt solution so that its concentration is above the normal 1.6 mM it will enhance a contractile response (Godfraind and Kaba, 1972). Calcium acts to initicate the contractile process by combining with troponin, which is a regulatory protein, and changing it in such a way that it is no longer able to inhibit the ATPase activity of actomyosin. Once the ATPase activity is derepressed free energy from ATP is released and made

available for contraction. The amount of free energy released and consequently the magnitude of contraction, is directly related to the concentration of calcium which is available to the contractile elements (Bohr and Sitrin, 1970). This calcium for contraction is available from two sources. It can come from the extracellular fluid where it may be as much as 1000 times more concentrated than intracellularly or it may be made available from some bound intracellular pool or membrane trap. Both sources of calcium are used in the course of a normal contraction.

If the similarity between cadmium and calcium ions is in fact the basis for some of the effects observed *in vitro* then perhaps this would also explain the acute *in vivo* responses to cadmium. A number of studies have shown that cadmium will produce an acute, prompt and marked hypertension when injected either IV or IP (Perry and Erlanger, 1971; Schroeder *et al.*, 1966). While there is a report that the acute cadmium induced hypertension is associated with increased cardiac output (Perry *et al.*, 1967) the direct effects of cadmium on arteries would appear to play an important role. The fact that both *in vivo* cadmium hypertension and *in vitro* aortic strip contraction are prevented and/or reversed by a cadmium chelating agent argues for a common mechanism of action in both situations. Because there was no response

to cadmium in a calcium free medium implies a further link between these two ions. It would appear that the contractile response seen after exposure of normotensive strips to cadmium is mediated via the calcium ion.

Very often after the normotensive aortic strips were exposed to cadmium they could not be made to relax back to the original baseline. This was noted despite many rinses with physiological salt solution. If they relaxed at all it was back to some point above the baseline. It appeared as though the cadmium had caused the resting tension to be reset at a higher level. Even after the aortic strips were then exposed to norepinephrine they then relaxed back to the new higher baseline.

Toda (1973) has reported on the acute effects of cadmium ions on isolated rabbit aortic strips. He measured the contractile responses to potassium, norepinephrine, histamine and angiotensin after a 20 minute exposure to either 0.1 or 0.5 mM cadmium ion. There was a decreased response to all of these agonists after cadmium exposure with the greatest decreases after the 0.5 mM cadmium concentration. Cysteine (1 mM) was also noted to have the ability to restore either partially or completely the contractile response after the cadmium induced inhibition. He also noted that in aortic strips which were depolarized by potassium in a calcium free medium, the contraction produced by the addition of

calcium was suppressed by the presence of cadmium. This suggests that cadmium is interfering with the inward flux of calcium which is known to play a key role in potassium contractions. This study made no mention of any direct effects, such as contraction, produced by exposure to cadmium ions.

Cadmium ions are known to form complexes with sulfhydryl groups. Compounds such as dimercaprol (BAL) have been used to treat cadmium poisoning in humans (Browning, 1961) and BAL and another thiol, cysteine have been demonstrated to prevent cadmium induced necrosis in the testicles of rats (Gunn et al., 1968). The specific cadmium binding protein metallothioneinwhich was mentioned earlier also complexes cadmium by virtue of its abundance of SH groups. Thus there is strong evidence that cadmium will very easily form chelates with sulfhydryl groups.

Needleman et al. (1973) have reported a sulfhydryl requirement for relaxation in vascular smooth muscle. They incubated rabbit aortic strips with ethacrynic acid which alkylates SH groups. After this procedure the vascular relaxation to all tested vasodilators was abolished while the tissue retained its normal contractility to KCl or norepinephrine. A number of compounds which have found clinical use as potent vasodilators (sodium nitroprusside and cystamine) are also known to be excellent SH oxidizers. These authors conclude that there are

critical SH sites which are intimately involved in the relaxation of vascular smooth muscle. Since cadmium is known to form complexes with SH groups, it is possible that this could be contributing to the relaxation defect seen when cadmium was added to normotensive aortic strips. This idea will be further explored in the following section.

F. Effects of $Na_2CaEDTA$ on Responses of Aortic Strips

Schroeder and Buckman (1967) were able to reverse cadmium induced hypertension by administering a specific cadmium binding chelating agent. This chelating agent, trans-cyclohexane-1,2-diamine-N,N,N',N'-tetraacetic acid (CDTA) has a higher affinity for cadmium than for zinc. It is important for *in vivo* work that zinc, which is a necessary co-factor for a number of reactions, is not disturbed. Rats which had been made hypertensive by either oral or intraperitoneal cadmium acetate were returned to normotensive levels within two weeks after one or more injections of disodium zinc CDTA. Presumably the tissue cadmium was exchanged for zinc and in this way the overall body burden of cadmium reduced.

Since the effects of chelation were so dramatic *in vivo* it seemed only natural to study the effects *in vitro*. To this end a number of experiments were carried out using the chelating agent $Na_2CaEDTA$. In these experiments it

was not the possible chelation of zinc which could be a problem but rather the calcium. Therefore the calcium salt was chosen to reduce the possibility of removing any calcium from the system. The effects of $Na_2CaEDTA$ were studied on both chronically and acutely cadmium exposed aortic strips.

A very interesting effect was seen when $Na_2CaEDTA$ was addeded to the physiological salt solution containing a hypertensive and normotensive strip. It caused a small ($\bar{x}$ = 125 mg) relaxation in the hypertensive strip and nothing at all in the normotensive strip. This finding would appear to support the idea that cadmium does in fact interfere with and raise the set point of vascular smooth muscle resting tone.

A further explanation for this observation might be the previously mentioned affinity which cadmium has for SH groups. If there are in fact specific SH groups which are involved with relaxation and cadmium ions have complexed with enough of them to prevent complete relaxation this could then explain a higher apparent passive tone. The $Na_2CaEDTA$ would chelate the cadmium away from these SH groups and the hypertensive aortic strip would relax back to a lower passive tension. The fact that the normotensive strips did not respond at all to this treatment lends further support to this idea.

The resting tone of vascular smooth muscle is to a large degree controlled by the availability of free or activator calcium to the contractile proteins. This calcium is in turn made available by either direct stimulation (norepinephrine) or by a constant leakage from various intracellular storage sites or membrane traps. If something caused an increase in the level of free calcium then the amount of resting tone should also be increased.

Some very interesting experiments were performed on rat diaphragm preparations by Hajdu et al. (1971) which showed that cadmium was bound more strongly to excitation-contraction sites than calcium. These experiments further showed that cadmium would block the uptake of calcium by these sites and could replace calcium in a 1:1 ratio. While these findings were not specifically derived from vascular smooth muscle, they support the idea that cadmium can either directly or indirectly affect the smooth muscle tone by virtue of its similarity to the calcium ion.

If, in fact, this replacement of calcium by cadmium is true in vascular smooth muscle, then a plausible explanation is possible for the development of cadmium hypertension. Since the amount of cadmium the animals were exposed to is low and since the amount which is absorbed is even lower, it takes months for enough cadmium to reach

the specific susceptible sites in the vascular smooth muscle. There are, of course, strong forces which work to keep the arterial system set on a normal resting tone. Over a number of months enough cadmium could find its way to the vascular smooth muscle cell membrane and by virtue of its similarity to the calcium ion would partially jam the calcium extrusion pump. This would displace enough calcium to raise the overall free calcium concentration. This in turn would stimulate enough extra contractile units to raise the overall level of vascular tone. Of course another explanation is that the cadmium acts directly on the contractile proteins and its effects are not mediated through calcium. It will be remembered that cadmium alone was able to produce a contraction which was almost equal in value to the amount that $Na_2CaEDTA$ was able to relax the cadmium hypertensive aortic strip. This latter point is seriously weakened by the fact that cadmium did not produce any response in a calcium-free medium. Support is therefore gained for the idea that cadmium exerts whatever effects it has through a calcium dependent mechanism. More support is also gained for the idea that cadmium is acting at the level of the cell membrane from the results of the EDTA studies. $Na_2CaEDTA$ is a large enough molecule that its permeability through

cell membranes would be very low. The fact that its effects were so rapid in several kinds of situations indicates indirectly that its point of action was predominantly extracellular.

There is growing evidence that there is an energy dependent (Mg-ATP) mechanism within vascular smooth muscle which is involved with calcium transport. This so-called calcium pump has been described by Fitzpatrick et al. (1972) and resides in a microsomal fraction of vascular smooth muscle. This system operates to control the availability of calcium within the cell and therefore plays a fundamental role in the maintenance of vascular tone. It is not necessary that a calcium pump actually remove calcium from the intracellular fluid. All that is necessary is for calcium not to be available in an ionic form which can interact with the contractile proteins.

Also noteworthy is the fact that $Na_2CaEDTA$ was able to prevent the cadmium induced contraction in the normotensive strip. The new higher resting tone or baseline so often seen after a normotensive strip was exposed to cadmium was also promptly reversed by the addition of $Na_2CaEDTA$ to the bathing medium.

It therefore seems possible that cadmium may be acting in two different, but complimentary, ways to increase vascular resistence. First, cadmium has been demonstrated to be able to cause a contraction in

vascular smooth muscle. This is a kind of *in vitro* confirmation of a previously reported pressor effect of cadmium *in vivo*. Second is the observed lowering of the resting tension in cadmium hypertensive aortic strips by a compound which chelates cadmium. There is some indirect evidence that this cadmium may have been interfering with specific SH groups which are necessary for smooth muscle relaxation.

Some of the most interesting results of the entire investigation were seen when the interactions between cadmium, $Na_2CaEDTA$, and norepinephrine were studied. It will be recalled that when cadmium preceeded norepinephrine in a normotensive strip the slow component of the contraction was prevented. The slow component could then be beautifully restored by the addition of Na2CaEDTA to the physiological salt solution. The slow component of the contraction (sometimes referred to as the tonic contraction) will also be prevented if a strip is stimulated in a calcium free solution. When norephinephrine produces a contraction in smooth muscle a typical two part response is seen. The norepinephrine initially triggers the release of bound or trapped intracellular calcium and this calcium produces the initial fast (or phasic) part of the contraction. It is during this time that the cell membrane becomes more permeable to extracellular calcium and some of this calcium leaks into the

cell thereby producing the slow component. Since this slow component is not seen in a calcium free solution and since the presence of cadmium ion will also prevent it, the only conclusion which can be reached is that cadmium has blocked the transport and/or the channels for extracellular calcium to enter the cell. This is further supported by the speed with which $Na_2CaEDTA$ can restore the slow component by chelating the cadmium ion.

A similar type of observation was made by Shibata et al. (1973) when they were studying the responses of aortic strips from spontaneously hypertensive rats. They noted that the slow component was greatly reduced or absent when aortic strips from spontaneously hypertensive rats were stimulated with either norepinephrine or potassium. Their tentative explanation for these findings was that there was some alteration in the cell membrane which prevented enough calcium from being available for the entire excitation-contraction coupling process. These experiments lend further support to the idea that cadmium will bind to or displace calcium from certain calcium binding sites on the cell membrane.

This slow component blockade which is produced by cadmium is only seen during an acute exposure. There was no evidence at all of any slow component defect in any of the aortic strips from hypertensive rats which had been chronically exposed to cadmium. Probably during a chronic

exposure cadmium reaches an equilibrium with whatever sites it is going to occupy. In this way both the fast and slow component are equally affected. Further support for the reversal of cadmium hypertension *in vivo* by CDTA is furnished by the following *in vitro* findings. First is the ease with which $Na_2CaEDTA$ can restore the slow component in acutely exposed strips as well as lower the resting tone in chronically exposed strips.

As a further check on the ability of cadmium to bind to certain calcium binding sites the lanthanum experiments were undertaken. Lanthanum is a trivalent cation which is known to have a higher affinity for smooth muscle calcium binding sites than calcium (vanBreeman *et al.*, 1972; vanBreeman *et al.*, 1973). These properties can be used to study the movement of calcium into and out of the muscle cell. Lanthanum will also block the slow component of the norepinephrine contractions by stopping the influx of extracellular calcium. This effect is almost impossible to reverse with EDTA as in the case of cadmium, because lanthanum is more firmly bound to these sites. Lanthanum would also displace cadmium from these sites because the slow component could not be recovered after treatment with both of these agents. The fact that lanthanum, which has a known affinity for these membrane calcium sites, also blocks the slow component is further confirmation for the explanation of how cadmium produces this same effect.

G. Effects of Cadmium on Vascular Smooth Muscle under Anaerobic Conditions

Vascular smooth muscle normally functions under aerobic conditions but does have the capacity to operate anaerobically. Energy for contraction under anaerobic conditions can be obtained through the process of glycolysis. There is some evidence that anoxia will lead to some modest decreases of the contractile response to drugs (Shibata and Briggs, 1973; Pittman and Duling, 1973). When cadmium was added to the physiological salt solution bathing aortic strips made anoxic by vigorous nitrogen bubbling through the medium, no contractile response was seen. There was also a diminished response to norepinephrine under anaerobic conditions confirming the observations of Shibata and others. Two explanations for these observations are possible. The first is that cadmium produces its contractile effects through some strictly oxygen dependent process. This might be related to an effect that cadmium amy have on the cell membrane to alter its calcium binding properties. On the other hand this lack of response to the contractile effects of cadmium under anaerobic conditions may simply be due to the observed modest decreases of vascular smooth muscle to drugs in general under these conditions. Since the contractile effects of cadmium are very slight anyway a

decrease in overall responsiveness could easily negate the usual cadmium contraction. This could easily be the case since, as noted above, the response to norepinephrine was also decreased under anaerobic conditions.

While not strictly applicable to the subject being discussed, mention should be made of the effects of cadmium on mitochondria and oxidative metabolism of pulmonary alveolar macrophages (Mustafa and Cross, 1971). These investigators noted that cadmium had the ability to uncouple oxidative respiration in the mitochondria of these cells. They further observed that the chelating agents EDTA and dimercaprol were able to almost completely reverse the inhibitory effects of cadmium on mitochondrial respiration. They offer the explanation that cadmium produces the respiratory inhibition by binding to certain components of the respiratory chain. These components they suggest, are the various thiol containing flavoproteins and dehydrogenases (α-ketoglutarate dehydrogenase) of the respiratory chain. This inhibition is reversible and does not appear to produce any permanent damage to the enzymes.

The significance of these findings to vascular smooth muscle is of course highly speculative. Cadmium has also been demonstrated to uncouple oxidative phosphorylation in liver mitochondria (Jacobs et al., 1956). Whether or not this same effect would be seen in smooth muscle

mitochondria is not known. If cadmium produced a partial or complete uncoupling of smooth muscle mitochondria, it seems as though there would be a decrease in the overall ability of the cell to function. This might then be translated to a decreased responsiveness of aortic strips to various agonists through impaired vascular smooth muscle mitochondria.

H. Tissue Cadmium Levels

Because a large number of animals were exposed to cadmium for a considerable length of time, the cadmium levels in various organs were determined. This was not done with the idea of discovering something new since this sort of thing has been done repeatedly, but rather to become familiar with certain analytical techniques as well as to try and correlate tissue levels with the effects produced by this metal.

As expected, there were large differences seen between exposed and control animals in the amount of cadmium in several organs. The results obtained in this study were in general agreement with the hepatic and renal cadmium concentrations seen by other investigators. It is difficult to make precise comparisons because of differences in dose and length of exposure.

Study	Mean Kidney Cd (ug/g)	Mean Liver Cd (ug/g)
Golden	41.54	23.61
Fisher et al. (1971)	35.64	33.36
Anwar et al. (1961)	33.82	10.42

There was a significant difference between males and females in the concentration of both renal and hepatic cadmium (measured as ug cadmium/gram tissue).

Also noted was a tendency (not significant) for the female rats to have higher blood pressures. This kind of observation was also made by Schroeder (1963) who noted that female rats retained more cadmium than males and also developed more hypertension than males. Even in a normally exposed human population, females have higher renal and hepatic cadmium levels than males (Lewis et al., 1972). The relationship between sex, cadmium accumulation and blood pressure is still much too uncertain even to discuss with any precision. The above observations are confirmed by the fact that the female rats in this study showed a significantly greater partial body burden when computed by body weight. Since there is a tendency in human populations for females to develop more hypertension than males, it does seem possible that such a difference would be seen when cadmium hypertension is produced in rats. These kinds of observations must be tempered by the difficulty in extrapolating from rats to humans and vice versa.

I. Pathology

The renal lesions observed in the cadmium exposed animals cannot be completely attributed to the cadmium since an almost equal percentage of the controls showed similar lesions. There is a certain amount of renal disease which will be seen as rats approach the end of their normal life span. Cadmium exposure did produce an excess of renal lesions as evidenced by the increased severity in more of the exposed animals. As with other aspects of cadmium toxicity there is considerable variability in the observations made on the possible effects of cadmium on renal morphology. Wilson et al. (1941) showed no glomerular damage and slight tubular changes after rats were exposed to 63 ppm cadmium chloride for 100 days. Princi and Geever (1950) exposed dogs to cadmium oxide dust (4 mg Cd/m^3) for prolonged periods and found no renal disease. When Dalhamm and Friberg (1957) injected cadmium chloride (0.65 mg/kg for six days) into rabbits they found mainly tubular changes and no glomerular lesions.

More recently Stowe et al. (1972) have described more extensive cadmium induced hepatic and renal lesions. They exposed rabbits to 160 ppm cadmium chloride in drinking water for six months. Morphological hepatic

findings included severe glycogen depletion and varying degrees of periportal and interlobular collagen deposition. They also noted biliary hyperplasia as well as inflammatory cell infiltrates in the portal regions. Renal disease included extensive interstitial fibrosis and various degrees of coagulative necrosis of the proximal tubules. No mention was made about any glomerular involvement.

In contrast to the above the present study did not reveal any hepatic disease. There was certainly ample opportunity for development of such disease as many of these animals were exposed for up to two years. The lack of any hepatic involvement only serves to confirm the previously mentioned variability in many aspects of cadmiun toxicity.

Even though the cadmium exposed and control animals showed almost equal incidence of renal disease, the exposed group exhibited a higher percentage of more severe lesions. In both groups there is more renal disease in the females than the males. In regard to this observation two previously mentioned points will be restated. The first is that there were higher blood pressures in the female rats as a group than the males. The second is that there is a higher incidence of Itai-Itai disease seen in multigravid postmenopausal women as compared to men. Whether or not there is a causal

relationship between the renal lesions and these facts is not known.

Another interesting observation was that animals exposed to cadmium in utero had a higher incidence of renal disease. The ability of cadmium to cross the placenta has already been discussed in reference to the higher level of hypertension seen in this same group of animals. Whether or not the hypertension and renal disease are related is not known. It might be speculated that the high percentage of renal lesions in rats exposed to cadmium before birth may be related to some as yet unexplored aspects of cadmium transport and metallothionein synthesis. Perhaps the synthesis of this specific cadmium binding protein is depressed in the fetus and it is therefore not available to protect the kidneys by virtue of its cadmium binding activity. More free cadmium would then be available to react with susceptible renal tissue.

Another point which should be made concerns the differences between the two groups in only the degree of renal disease but the similarity in kind. Since the control rats showed similar kinds of renal lesions as the cadmium exposed it might be interpreted that all observed lesions were only the result of the normal ageing process. It could be that cadmium accelerates the process or inhibits whatever mechanisms which usually act to repair this type of renal damage. The fact that identical renal

damage was seen in both groups would seem to support this observation.

CONCLUSION

This study was undertaken in an effort to elucidate a possible mechanism of action for the production of arterial hypertension by cadmium. Hypertension was produced by either oral or IP administration of cadmium chloride. Its effects on the vascular smooth muscle of the thoracic aorta were then studied. Aortic strips from hypertensive rats were less responsive to the contractile effects of norepinephrine, serotonin, and potassium chloride than controls. Differences in stiffness did not appear to play a part in this decreased responsiveness. These observations are consistent with similar experiments done on aortic strips from animals whose hypertension was produced in different ways. Since cadmium (10^{-4} M) produced a contraction in the control strips and no response in the hypertensive strips it was concluded that whatever effect cadmium might have on vascular smooth muscle had already occurred in vivo. This was partially confirmed by the studies done using the chelating agent $Na_2CaEDTA$. This compound was found to prevent or reverse a cadmium contraction in the control strips as well as decrease the resting tension (or passive tone) of a cadmium hypertensive strip. These effects were

probably mediated at the cell membrane, because EDTA would not penetrate the cell easily and certainly not in the short time these effects were observed. Cadmium was also able to prevent the slow component of a norepinephrine contraction in an acutely exposed aortic strip. This inhibition could be promptly reversed at the plateau of the contractions by the addition of EDTA. None of the effects of cadmium were seen when calcium was absent from the bathing medium.

On the basis of these kinds of observations it was concluded that cadmium, at least in the acute situation, was producing its effects by interfering with the movement of calcium across the cell membrane. This was confirmed by the lanthanum experiments which showed that a substance with a known high affinity for calcium binding sites would also produce similar effects to cadmium.

Whether this explanation is adequate in the chronic situation is not as certain. There can be little doubt that cadmium has interfered at the membrane level since EDTA produced a relaxation in the hypertensive aortic strip. This relaxation, however, could not account for the differences seen between the strips in response to various agonists. It seems possible that over a long time period cadmium may enter the smooth muscle cell and alter calcium dynamics in a way which is comparable to

its effects on the cell membrane. Also to be considered is the idea that cadmium may be interacting with critical SH groups which are necessary for proper relaxation of the muscle. This might also be supported by the EDTA relaxation of the cadmium hypertensive strips.

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