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Received August 24, 1966. P.S.E.B.M., 1967, v124.

In vitro Production and Inhibition of Aortic Vasoconstriction by Mercuric, Cadmium, and Other Metal Ions.* (31770)

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Vasoactivity of the subgroup II elements, cadmium and mercury, has been demonstrated in three ways: First, intra-arterial injections of less than 0.05 mg of either element into anesthetized rats have produced acute, but transient increases in diastolic pressure(1). Although the pressor effects of the two ions were superficially similar, the mercuric ion did not alter cardiac output, whereas the cadmium ion increased it proportionally to the increase in pressure. Second, rats have exhibited prolonged hypertension after drinking water containing 5 parts per million of cadmium for several months(2) or following several weekly intraperitoneal injections of 0.2 mg of cadmium(3). Finally micromolar concentrations of mercuric ion have induced single slow contractions in spirally-cut strips of rabbit aorta, as described here. While cadmium ion produced no similar effect, it inhibited the effect of mercuric ion. It also inhibited epinephrine-induced, but not angiotensin-induced, contractions of aortic strips.

Methods. Aortic strips were set up according to the technique described by Furchgott and Bhadrakom(4) and modified in our laboratory(5): A large normal unanesthetized female New Zealand white rabbit was decapitated, its descending thoracic aorta quickly removed, trimmed of excess fat and connective tissue, and cut into four spiral strips approximately 2 mm wide and 25 mm long, with circumferential muscle fibers making an angle of about 15 degrees with the long axis of the strip. Each strip was mounted in an individual chamber where it was covered completely by 20 ml of Krebs Ringer bicarbonate solution† to each 100 ml of which 200 mg of glucose had been added. The temperature of the solution was maintained at 37 ± 0.1°C, and 100 ml per min of 95% O_2 and 5% CO_2 was continually bubbled through it. The bottom of a strip was fixed and the top attached to the lever of an ink-writing, gravity-type pen which amplified contractions 4-fold. The lever was counterweighted to exert constant tension of 1.7 g on a strip which was mounted so that, after 2 hours when it had completely relaxed, about 20 mm separated the top and bottom points of attachment. The responsiveness of each strip was tested by exposing it to 10^{-7} molar l-epinephrine and recording the maximum contraction. The solution containing epinephrine was then removed and replaced with fresh Krebs Ringer bicarbonate solution; this washing was repeated at 5-minute intervals until, after 15 to 30 minutes, the strip had completely relaxed and regained its previous responsiveness. Except for standardization with epinephrine, no strip was exposed to more than one metal or other test agent unless specifically indicated. All cited molarities apply to concentrations in the solution surrounding the strip.

Results. Approximately 20% shortening of the aortic strip was produced by 10^{-7} molar

* Supported by USPHS Grants OH 106 and HE 2577.

† The Krebs Ringer bicarbonate solution was composed of 100 parts of 0.90% (by weight) NaCl, 4 parts of 1.15% KCl, 3 parts of 1.22% $CaCl_2$, 1 part of 2.11% KH_2PO_4, 1 part of 3.82% $MgSO_4 \cdot 7H_2O$, and 21 parts of 1.30% $NaHCO_3$, all dissolved in deionized distilled water.

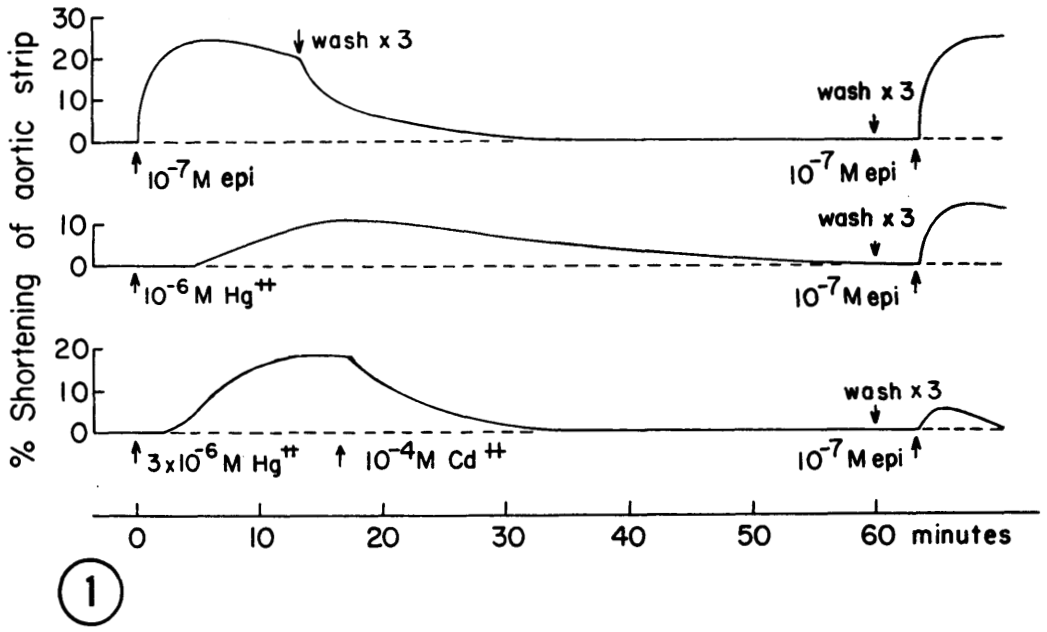

FIG. 1. Typical responses of 3 aortic strips from the same rabbit to: (a) 10^{-7} M l-epinephrine twice. *Note* similarity of the two curves. (b) 10^{-6} M Hg^{++} followed by 10^{-7} M l-epinephrine. *Note* slow constant rate of metal-induced contraction and subsequent lessened contraction with epinephrine. (c) $3x10^{-6}$ M Hg^{++} followed by 10^{-4} M Cd^{++} and later 10^{-7} M l-epinephrine. *Note* immediate and abrupt cadmium-induced relaxation and subsequent diminished contraction with epinephrine. (The diminution of the epinephrine-induced contraction was the same following this concentration of mercury without cadmium).

l-epinephrine; whereas 10^{-6} molar mercuric ion‡ produced approximately 10% shortening. The metal-induced contraction was slower and its onset was delayed (Fig. 1a and b). In addition its rate of shortening was relatively constant, while the epinephrine-induced contraction began very rapidly and then became slower. The difference in the shape of the types of curves traced by the contracting strips is emphasized by evaluating the following fraction for both:

$$\frac{\text{Time for half maximal contraction}}{\text{Time for maximal contraction}}$$

The numerical value of this fraction varied from 0.3 to 0.5 for 12 strips exposed to 10^{-6} molar mercuric ion and from 0.1 to 0.2 for 12 strips exposed to 10^{-7} molar l-epinephrine. Even without washing, the metal-induced contraction was followed by spontaneous relaxation of the strip to its original length, after which it was refractory to mercury.

The degree of shortening of the strip was a function of the concentration of mercuric ion, varying from 2.5% with 10^{-7} to 21% with 10^{-4} molar mercuric ion. The numerical value of the fraction cited above, however, was not altered by this thousand-fold change in concentration of mercuric ion. The latent period between the exhibition of mercury and the onset of contraction, the period required for maximal contraction, and the subsequent period for 50% relaxation were all approximately eight times as long for 10^{-7} molar as for 10^{-4} molar mercuric ions, with the times for 10^{-4} molar mercuric ion being 1.0, 6.8, and 11 minutes for the 3 periods respectively. Mercury dimethyl and meralluride at concentrations of 10^{-5} molar had no evident effect on the strip.

Neither zinc nor cadmium ions, at concentrations as high as 10^{-4} molar, regularly induced contraction of aortic strips, although

‡ The term mercuric ion is used for convenience despite the fact that the concentration of free Hg^{++} in Krebs Ringer bicarbonate solution was very small because of the excess chloride ion which binds the metal in complexes which may be anionic.

TABLE I. Metal-Induced Contraction of Aortic Muscle.

Ion	Concentration (molar)	Shortening of strip (%)
Hg^{++}	10^{-6}	9
Hg^{++}	3×10^{-6}	16
Hg^{++}	10^{-5}	18
Hg^{++}	10^{-4}	21
Ag^{+}	10^{-6}	0
Ag^{+}	3×10^{-6}	23
Ag^{+}	10^{-5}	23
Ag^{+}	10^{-4}	25
Cu^{++}	10^{-5}	6
Cu^{++}	10^{-4}	8
Cu^{+}	10^{-4}	7
Ba^{++}	10^{-4}	5
Hg_2^{++}	10^{-4}	5
VO^{++}	10^{-4}	4

Each value cited is the average obtained by using at least 3 strips from each of 2 rabbits. Each strip was exposed serially to 10^{-6}, 3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} molar concentrations of one metal, the next higher concentration being added as soon as maximum contraction had occurred or after 15 minutes if there was no visible contraction. In each case the first average contraction of 2% or more is tabulated.

on a few occasions 10^{-4} molar cadmium ion induced a small slow contraction after a latent period of more than 10 minutes. Cadmium, but not zinc ions, consistently blocked the contractions induced by smaller concentrations of mercury: 10^{-4} molar cadmium ion partially inhibited one-tenth, and almost completely inhibited one-thirtieth, as much mercuric ion. The effect was prompt (Fig. 1c) and equally marked whether the cadmium ion was exhibited before or after the mercuric ion.

The contractions induced by l-epinephrine and angiotensin II were prolongedly inhibited by mercuric ion, the inhibition being proportional to the magnitude of the mercury-induced contraction: The vasoconstrictor effect of 10^{-7} molar l-epinephrine was little affected by the previous exhibition of 10^{-7} molar, approximately halved by 10^{-6} molar, and almost completely blocked by 10^{-5} molar mercuric ion. Mercuric ion comparably inhibited the contraction induced by 2×10^{-8} molar 1-L-asparaginyl-5-L-valyl (Fig. 2a). Inhibition of both substances by mercury persisted unchanged for more than 2 hours. Without itself altering the length of the strip, cadmium inhibited the contraction induced by l-epinephrine but not by angiotensin II: Against l-epinephrine cadmium, exhibited for from 1 to 15 minutes, was about one-tenth as effective as mercury, and the effect was similarly prolonged. In contrast even 10^{-4} molar cadmium enhanced rather than inhibited angiotensin II (Fig. 2b). Zinc in concentrations as high as 10^{-4} molar did not obviously affect either l-epinephrine or angiotensin II (Fig. 2c).

A search for other vasoactive metals revealed none that was effective in concentrations as low as 10^{-6} molar, but concentrations between 3×10^{-6} and 10^{-4} molar of silver, cupric, cuprous, barium, mercurous, and vanadyl ions produced significant contractions of aortic strips. The time course of these contractions is shown in Fig. 3 and their magnitudes are indicated in Table I. Other metallic ions tested and found to be inert at concentrations between 10^{-6} and 10^{-4} molar, included Cr^{++}, Mn^{++}, Fe^{++}, Co^{++}, Ni^{++}, Sn^{++}, Pb^{++}, Al^{+++}, Ti^{+++}, Cr^{+++}, Fe^{+++}, and Au^{+++}.

Prior exhibition of 10^{-4} molar n-ethylmaleimide and p-chloromercuribenzoate, both of which are sulfhydryl-binding agents, markedly reduced the contraction produced by 10^{-6} molar mercuric ion, but did not affect that produced by epinephrine. Addition of 10^{-7} molar ethylenediamine tetra-acetate (EDTA), a chelating agent used as the calcium disodium salt in order to avoid binding the alkali metals and the alkaline earths in the solution bathing the strips, almost doubled the mercury-induced shortening, while 10^{-3} molar EDTA inhibited it slightly. Cyanide, hydralazine, and phentolamine were all inhibitory (Table II). Aortas from rabbits which had received 5 mg of reserpine per kg 24 hours before sacrifice in order to release bound catecholamine, responded to mercuric ions in the same manner as aortas from untreated rabbits.

Discussion. The data presented here describe metal-induced contractions of vascular smooth muscle. The mechanism of the effect remains entirely speculative. It may involve the binding of sulfhydryl groups. The mercuric ion is bound strongly by such groups, and

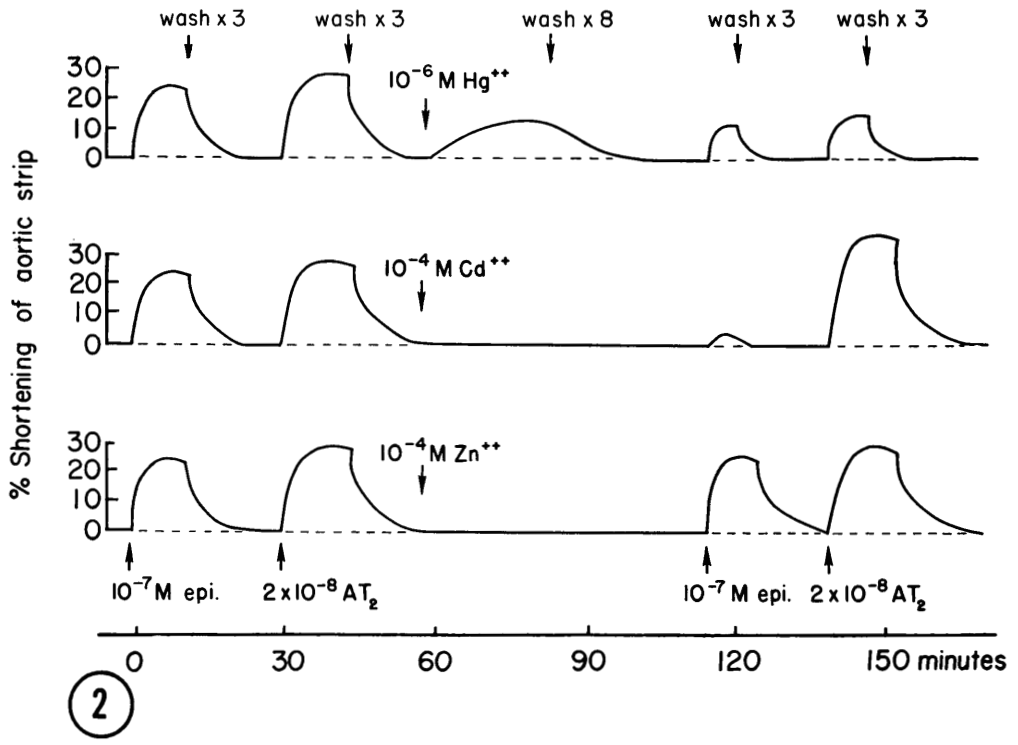

FIG. 2. Typical responses of 3 aortic strips from the same rabbit to 10^{-7} M l-epinephrine and then 2×10^{-8} M angiotensin II before and after exposure to (a) 10^{-6} M Hg^{++}; 10^{-4} M Cd^{++}; (c) 10^{-4} M Zn^{++}. *Note* the inhibition of both vasopressor agents by Hg^{++}, of one by Cd^{++}, and of neither by Zn^{++}.

mercury-induced contractions were observed to be inhibited by sulfhydryl-binding agents. Sulfhydryl groups are present in the muscle protein, actin, and Kuschinsky and Turba first postulated their involvement in the polymerization of G-actin to F-actin after noting inhibition of that polymerization by mersalyl(6). Since then, reduction in the number of free sulfhydryl groups during the polymerization has been both affirmed(7) and denied(8). More recently Blum has cited evidence for the involvement of two different kinds of sulfhydryl groups in the adenosine triphosphatase activity of myosin (9) and in the functioning of actomyosin(10). He also described enhancement of the ATPase activity by low concentrations of p-chloromercuribenzoate and cupric, cadmium and zinc ions as well as inhibition by high concentrations of the same substances(9).

The contraction of protein threads from sea urchin eggs under the influence of metallic ions(11) presents an interesting parallel to the contraction of vascular smooth muscle reported here. Even the unexplained enhancement by EDTA of mercury-induced contractions of aortic strips finds a parallel in the behavior of the protein from sea urchin, although in the latter system EDTA did not enhance the effect of any but the tri-valent ions of chromium, iron, and aluminum and the tetra-valent ion of tin(11).

The interrelationship of mercury, cadmium, epinephrine, and angiotensin were interesting and suggested that cadmium was an adrenergic blocker but that the pharmacologic effect of mercury was quite different. While mercury induced a contraction, it could do so only once. Cadmium, on the other hand, did not induce contractions, but it did block subsequent mercury-induced contractions and reverse already existing ones, the reversal being surprisingly rapid. Both cadmium and mercury irreversibly inhibited epinephrine,

but only mercury inhibited angiotensin.

Summary. Low concentrations of mercuric ion induced slow steady contractions of spirally-cut strips of thoracic aorta from normal rabbits. The shortening of the strip was 2.5% with 10^{-7} molar and 21% with 10^{-4} molar mercuric ion. Such a contraction was preceded by a latent period and followed by spontaneous relaxation, after which the strip was refractory to the original stimulus. Cadmium ions did not induce contractions, but did inhibit those induced by lower concen-

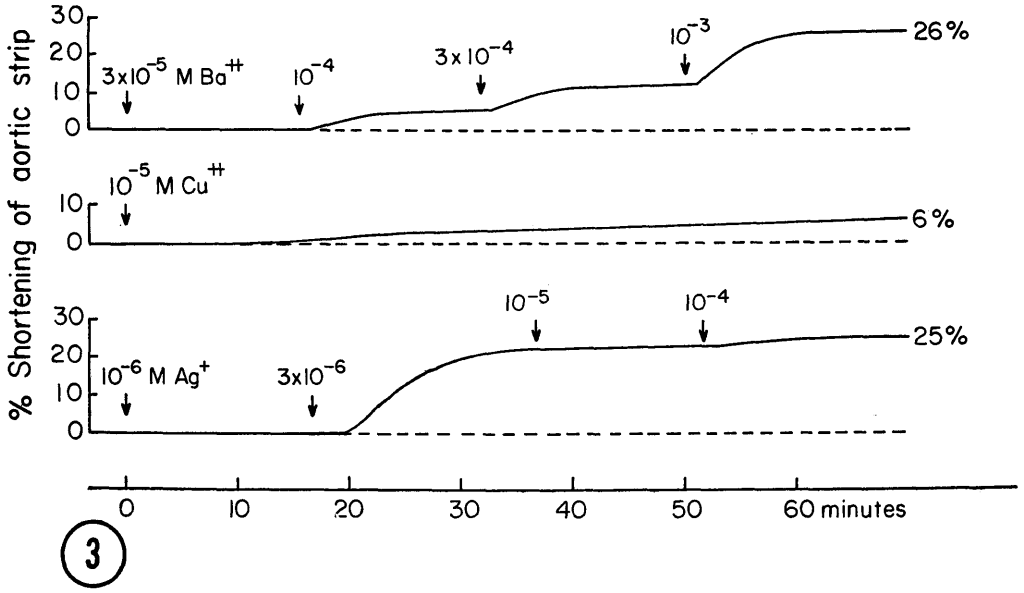

FIG. 3. Typical responses of 3 aortic strips from the same rabbit to: (a) Increasing concentration of Ba^{++}. *Note* except for the short latent period, shapes of these curves and their temporal relationships resemble those induced by 1/100 as much Hg^{++}. (b) 10^{-5} M Cu^{++}. *Note* very slow contraction resembled that induced by 10^{-7} M Hg^{++}, but increasing the concentration of Cu^{++} did not greatly alter rate or magnitude of contraction. (The 6% contraction obtained after 70 min was maximal). (c) Increasing concentrations of Ag^{+}. *Note* "all-or-none" response. Shape of the curve and its temporal relationships resembled those induced by a similar concentration of Hg^{++}. Both Hg_2^{++} and VO^{++} produced contractions of the Hg^{++} type, while Cu^{+} produced contractions of the Cu^{++} type. The anions used, $SO_4^{=}$ and Cl^{-}, produced no effect at concentrations of 10^{-3} molar.

TABLE II. Effect of Metal-Binding, Sulfhydryl-Binding, and Vasoinhibitory Substances on Mercury-Induced Contractions of Aortic Muscle.

Molarity of added material	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
	Fraction of expected shortening from 10^{-6} M Hg^{++}					
$CaNa_2EDTA$	0.72	1.00	1.38	1.76	1.86	1.00
N-ethylmaleimide	0.16	.36	.82	.91	1.03	
P-chloromercuribenzoate	0	.40	.68	.83	1.02	
Cyanide	0	.15	.25	1.10	.96	
Hydralazine	0	.61	.80	.97		
Phentolamine	0	0	.39	1.03		

After routine standardization, each strip was exposed to one of the indicated concentrations of one of the 6 compounds listed. Then after 5 minutes, the strip was exposed to 10^{-6} molar Hg^{++} as well. Since there were no washings, the first compound was still present when the mercury was added. Each tabulated value represents the fraction of the "expected" shortening from 10^{-6} molar Hg^{++} and is the average obtained by using at least 2 strips from each of 3 rabbits. To determine the "expected" response to Hg^{++}, 2 strips from each rabbit were exposed to 10^{-6} molar Hg^{++} alone.

trations of mercuric ions. Both mercuric and cadmium ions inhibited l-epinephrine; mercuric, but not cadmium, ions inhibited angiotensin II. Zinc ions were inert. Silver, cupric, cuprous, barium, mercurous, and vanadyl ions induced contractions but only in concentrations greater than 10^{-6} molar. Relatively high concentrations of two sulfhydryl-binding agents inhibited contractions induced by the mercuric ion; whereas low concentrations of a chelating agent markedly augmented them.

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Received September 9, 1966. P.S.E.B.M., 1967, v124.

Effect of Cortisone on the Lipids of Bone Matrix in the Rat.* (31771)

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Recent studies have shown that lipids are present in significant amounts in the matrix of bone and that they vary upon the administration of exogenous agents which alter the state of mineralization of bone(1,2). Because of the known effect of cortisone on bone(3,4) and because cortisone is known to alter systemic lipid metabolism(5) the following experiments were carried out in order to determine whether the lipids of bone matrix are changed after cortisone administration and whether the osseous changes brought about by cortisone can be related to lipid changes.

Material and methods. 150 g male R.V.H. strain rats were divided into 4 groups of 10 each, as follows: (1) control, (2) adrenalectomized, (3) cortisone treated, and (4) adrenalectomized and cortisone treated.

The adrenal glands of the rats from Groups 2 and 4 were removed under light ether anaesthesia through a posterior approach. One-half of Groups 1 and 3 were subjected to a false operation in which all steps were carried out except removal of the gland. The remainder of the animals of these groups served as an absolute control.

Six hours after operation 2 mg of cortisone acetate per .2 ml (Cortone, Merck, Sharp Dohne Ltd.) per 100 g body weight was injected subcutaneously to Groups 3 and 4. The same amount of the suspension base was given to the rats of Groups 1 and 2. Injections were given every day at 24-hour intervals for 10 days. Each cage of experimental animals consisted of 5 rats. They were allowed to eat Purina Labena and to drink tap water *ad libitum* except for the rats of Group 2, to which 1% NaCl solution was given. Body weight, and food and water consumption per 24 hours were recorded. Twenty hours after the last injection all animals were anaesthesized with intraperitoneal nembutal and blood was collected from the abdominal aorta. Complete removal of the glands was ascertained at this time. After sacrifice, the bones were removed and weighed.

Serum calcium concentration was determined according to the method of Yanagisawa (6). The humeri, femora, and tibias were removed from all animals and the soft tissues

* Supported by Grant MA-1571 from Medical Research Council of Canada.