Low concentrations of inorganic mercury inhibit in vitro autonomic transmission in the presence of albumin

Lars-Erik Moberg, Björn Appelgren and Nils O. Sjöstrand
Department of Prosthodontics and Department of Physiology, Karolinska Institutet, Stockholm, Sweden

Moberg L-E, Appelgren B, Sjöstrand NO. Low concentrations of inorganic mercury inhibit in vitro autonomic transmission in the presence of albumin. Acta Odontol Scand 1991;49:351–359. Oslo. ISSN 0001–6357.

The influence of albumin, 4.5 and 45 g/l, on the effects of Hg^{2+}, 10^{-9}–10^{-3} M, on the neuromuscular transmission of the isolated guinea-pig ileum and vas deferens was investigated. Hg^{2+}, 10^{-9}–10^{-6} M, transiently increased the basal tone of the ileum in Tyrode solution without albumin. Albumin, 4.5 g/l, reversed this stimulant effect but enhanced the contractile response to direct muscle stimulation. This contractile response also increased in the vas deferens. Albumin, 45 g/l, obliterated the stimulant effects of Hg^{2+} on the smooth muscle of the ileum but not of the vas deferens. The effects caused by higher concentrations of Hg^{2+}, 10^{-5}–10^{-4} M, were only partly inhibited when albumin was present. When neurogenic contractions were elicited in the presence of albumin (45 g/l), Hg^{2+}, 10^{-9}–10^{-4} M, reduced the contractions in both organs. Consequently, Hg^{2+} in concentrations presently considered acceptable in blood plasma (10^{-9}–10^{-8} M) suppressed both cholinergic and adrenergic neuromuscular transmission even in the presence of albumin. □ *Neuromuscular transmission; ileum; mercury; smooth muscle; vas deferens*

Lars-Erik Moberg, Department of Prosthodontics, Karolinska Institutet, Box 4064, S-141 04 Huddinge, Sweden

During the past decade increasing numbers of dental patients in Sweden have claimed that slow release of mercury from amalgam restorations causes various symptoms, such as pain, dizziness, tinnitus, taste and smarting sensations, nausea, tremor, paresthesia, and cardiovascular and respiratory symptoms (1–6). Recent studies have shown a continual release of mercury from amalgam restorations (7). Corrosion products from amalgam restorations are released into the saliva (8) and could, after swallowing, be absorbed through the gastrointestinal mucosa (9, 10). Mercury from amalgam restorations may also evaporate (11–14) and be absorbed through the pulmonary alveoli into the blood. The mercury binds to sulfhydryl groups in the erythrocytes and to plasma proteins, and simultaneously the oxidation stage is changed from Hg^0 to Hg^{2+} (mercuric mercury). Mercury from amalgam restorations may also be absorbed through the oral mucosa, the tooth root, and the surrounding bone (9–10). In the body inorganic Hg^{2+} compounds are bound mainly to proteins.

The average concentration of mercury in blood in a rural population is less than 2.5×10^{-8} M (5 ng/ml) (15) and comprises both inorganic and organic Hg^{2+} compounds. The contribution of mercury released from amalgam restorations to the Hg concentration in blood and urine has been a matter of dispute (6, 12, 16–18). A recent study showed a 50% decrease of the plasma Hg concentration, from 4.7 to 2.3×10^{-9} M, after removal of amalgam restorations (19). When amalgam fillings containing radioactive ^{203}Hg were placed in the teeth of sheep (9, 20) and monkeys (10), the whole blood concentration of the isotope was 1.5–4.5×10^{-8} M (range, 3–9 ng/g) and 3×10^{-8} M (5.8 ng/g), respectively. The ^{203}Hg was found in various organs of the animals,

such as skeletal muscle, heart, stomach, intestine, glands, and brain, in concentrations often higher than those in blood. In humans there also appears to be a positive correlation between the number of amalgam restorations in the oral cavity and the mercury concentration in the brain (21).

A previous study (22) showed that inorganic Hg^{2+}, in very low concentrations of 10^{-8}–10^{-6} M (2–200 ng/ml), affected cholinergic neuromuscular transmission in the guinea-pig ileum. It has been suggested that proteins may reduce the effects of metals (23). Consequently, we decided to investigate whether albumin, the main plasma protein, prevented or otherwise changed the effects exerted by inorganic Hg^{2+}, 10^{-9}–10^{-3} M, on the isolated guinea-pig ileum. For comparison, the effects of Hg^{2+} on the isolated guinea-pig vas deferens, supplied predominantly by adrenergic nerves (24), was also investigated.

Materials and methods

Mottled male guinea-pigs (250–500 g) were killed. Segments (2–3 cm) from the distal ileum and the pair of vas deferens were removed. The preparations were suspended between two parallel platinum electrodes (10 mm apart) in organ baths (4.4 ml) by means of surgical silk threads tied to the ends and attached to platinum hooks at the bottom of and above the organ baths. The hook above the bath was connected to a recording transducer (Grass FT 03C) coupled to a polygraph (Grass model 7B). The bath solution, a total of 44 ml, was recirculated (2 ml/min) in a closed system (25) and aerated with 6.5% CO_2 in O_2. The temperature was maintained at 37 °C. Before the start of experiments the preparations were allowed to equilibrate in the bath solution for at least 60 min. In the first series of experiments the effect of Hg^{2+} on the preparations immersed in ordinary Tyrode solution was studied. The composition of Tyrode solution was 136.7 mM NaCl, 2.7 mM KCl, 11.9 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.3 mM $NaHPO_4$ (all analytical grade, E. Merck) and 5.6 mM glucose (BDH) dissolved in deionized and double-distilled water. The pH in the aerated Tyrode solution was 7.4, and the concentration of free Ca^{2+}, 1.5 mM (measured by an ionized calcium analyzer, Nova 2, Nova Biomedical Corp., Newton, Mass., USA).

In a second series of experiments the effect of albumin was tested by changing the bath solution to fresh Tyrode solution containing 4.5 g/l or 45 g/l of albumin (Bovine, Fraction V, Sigma). By applying $NaHCO_3$ to the albumin solution to concentrations of 12.5 and 17.9 mM, respectively, pH was adjusted to 7.4. To maintain the concentration of ionized Ca^{2+} at 1.5 mM, $CaCl_2$ had to be increased to 2.0 and 3.7 mM, respectively.

A third series of experiments was performed to study the effect of increasing concentrations of $NaHCO_3$ in the Tyrode solution. $NaHCO_3$, 6 mM, was added to the Tyrode solution to achieve 17.9 mM, and pH was adjusted to 7.4 by titration with 1.2 M HCl (analytical grade, E. Merck). The $CaCl_2$ concentration was reduced from 1.8 to 1.6 mM to keep the ionized Ca^{2+} at 1.5 mM.

Transmural stimulation of nerves in the ileum was performed by single square-wave pulses of 0.5 msec duration, delivered from a Grass S44 stimulator every 48 or 60 sec with a strength of 60–180 mA, giving about half the maximum contractile response. The neurogenic nature of the response was verified by its sensitivity to tetrodotoxin (TTX) (0.3 μM). The vas deferens was stimulated by 3–10 Hz for 3 sec every 60 sec.

When direct smooth-muscle stimulation was performed, 0.3 μM TTX was added to the organ bath, and contractions were elicited by square-wave pulses with a duration of 2–20 msec, using 80–200 mA and 2–25 Hz, in the ileum for 1 sec and the vas deferens for 3–5 sec.

Fresh stock solutions were prepared with $HgCl_2$ dissolved in the bath medium, which was added to an initial concentration of 10^{-9} M (0.2 ng/ml) in the bath solution. The bath solution was pumped through the organ bath, and the action of Hg^{2+} on the contractility of the preparations was recorded. The criterion for an effect clearly attributable to Hg^{2+} was a change in response to electric stimuli more than 20% of the initial

Table 1. Effects of Hg^{2+} (in molar) on the basal tone and contractile response of isolated guinea-pig ileum and vas deferens in the presence of albumin

	Ileum								Vas deferens							
	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	*n*	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	*n*
Basal tone																
Tyrode solution	+	+	++	++	++	+++/−−−	+++/−−−	30	0	0	0	0	0	+++	+++	17
+ Albumin, 4.5 g/l	0	0	0	0	0	+++	+++/−−−	18	0	0	0	0	+	+++	+++	17
+ Albumin, 45 g/l	0	0	0	0	0	0	+++	28	0	0	0	0	0	0	+++	13
Muscle stimulation																
Tyrode solution	0	0	0	0	−−	−−−	−−−	20	0	0	0	+	+	++	+++	8
+ Albumin, 4.5 g/l	0	+	+	++	++	++/−−−	++/−−−	6	0	+	+	++	++	++/−−	++/−−−	11
+ Albumin, 45 g/l	0	0	0	0	0	0	−−−	8	0	+	+	+	++	++	+++/−−−	7
Nerve stimulation																
Tyrode solution	0	0	0	0	−−	−−−	−−−	10	+	+	+	++	++	++/−−	++/−−−	9
+ Albumin, 4.5 g/l	0	0	0	0	0	−−−	−−−	12	+	++	++	++	++	++/−−	++/−−−	6
+ Albumin, 45 g/l	0	−	−−	−−	−−	−−−	−−−	20	−	−−	−−	−−	−−	−−	+++/−−−	6

Increase is indicated by +, decrease by −, and no effect by 0. Preparations responding to increasing concentration of Hg^{2+} are cumulatively given as + (< 25% of the preparations), ++ (25–75%), +++ (> 75%), − (< 25%), −− (25–75%), −−− (> 75%). +/− indicates increase followed by decrease.

contractile response. The concentration of $HgCl_2$ in the organ bath was repeatedly increased 10-fold by additions of 0.2 ml of $HgCl_2$ solutions. The concentration of ionized Ca^{2+} in the bath solution was followed throughout the experiment and was stable around 1.5 mM. Effects of hyperosmolarity were examined by comparing equiosmolar concentrations of $HgCl_2$ and NaCl. Effects of $CuCl_2$ on the guinea-pig ileum and vas deferens were also studied to ascertain whether the effects seen were general divalent cation effects or specific for the elements. The effects of Cu^{2+} are presented in a following paper (26). Preparations with high spontaneous activity were excluded from the study. Each type of experiment was performed on 6–30 preparations. Four ileum and two vas deferens preparations were taken from each guinea-pig.

Results

Table 1 summarizes the effects of increasing concentrations of Hg^{2+} on the basal tone and contractile response to electric stimulation of the ileum and the vas deferens. No effects were observed when NaCl was added to the bath in equiosmolar concentrations.

Response of the smooth muscle

Effects of Hg^{2+} on the basal tone and on the contractile response to direct muscle stimulation. Hg^{2+}, 10^{-9}–10^{-6} M, transiently (5–10 min) raised the basal tone of the ileum in ordinary Tyrode solution (Fig. 1A), whereas in the vas deferens a slight enhancement on the contractions induced by electric stimulation was seen (Hg^{2+}, 10^{-6}–10^{-5} M) (Fig. 2A). Higher concentrations of Hg^{2+},

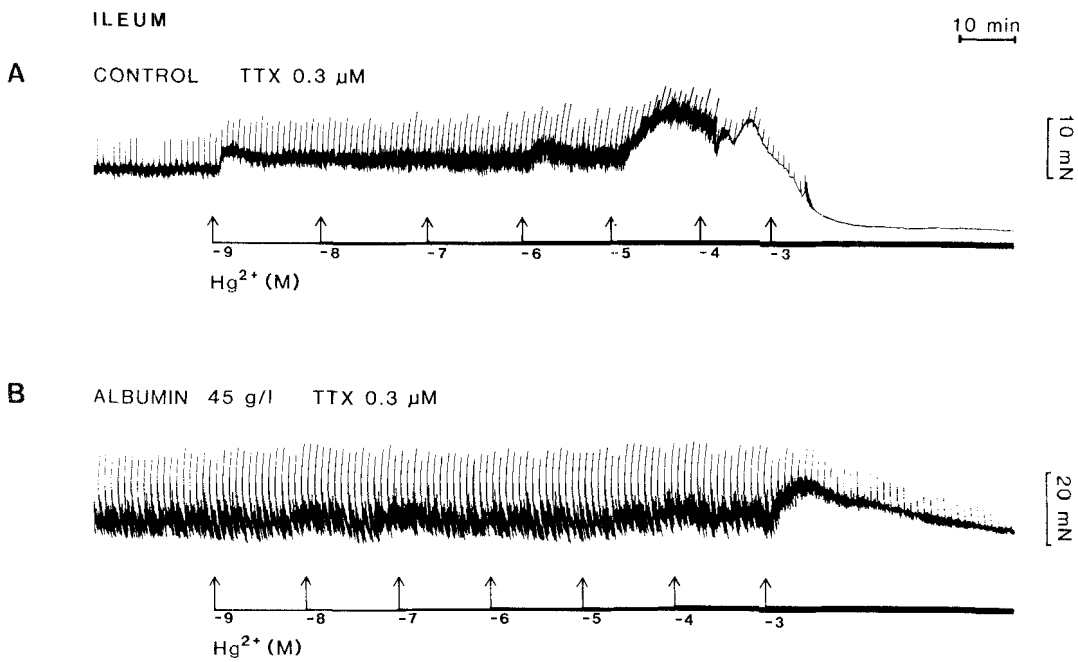

Fig. 1. Hg^{2+} effects in concentrations of 10 logarithms on direct muscle-stimulated guinea-pig ileum. The neurogenic response of stimulation was blocked with 0.3 µM tetrodotoxin (TTX). 1A. Hg^{2+} increased the basal tone at concentrations of 10^{-9} and 10^{-6} M. At concentrations of 10^{-5} and 10^{-4} M the basal tone sharply increased, and the response to direct muscle stimulation was depressed. 1B. Albumin at a concentration of 45 g/l inhibited the effects of Hg^{2+} at low concentrations and counteracted the rise in tone of the ileum, which did not occur until 10^{-3} M Hg^{2+} was added.

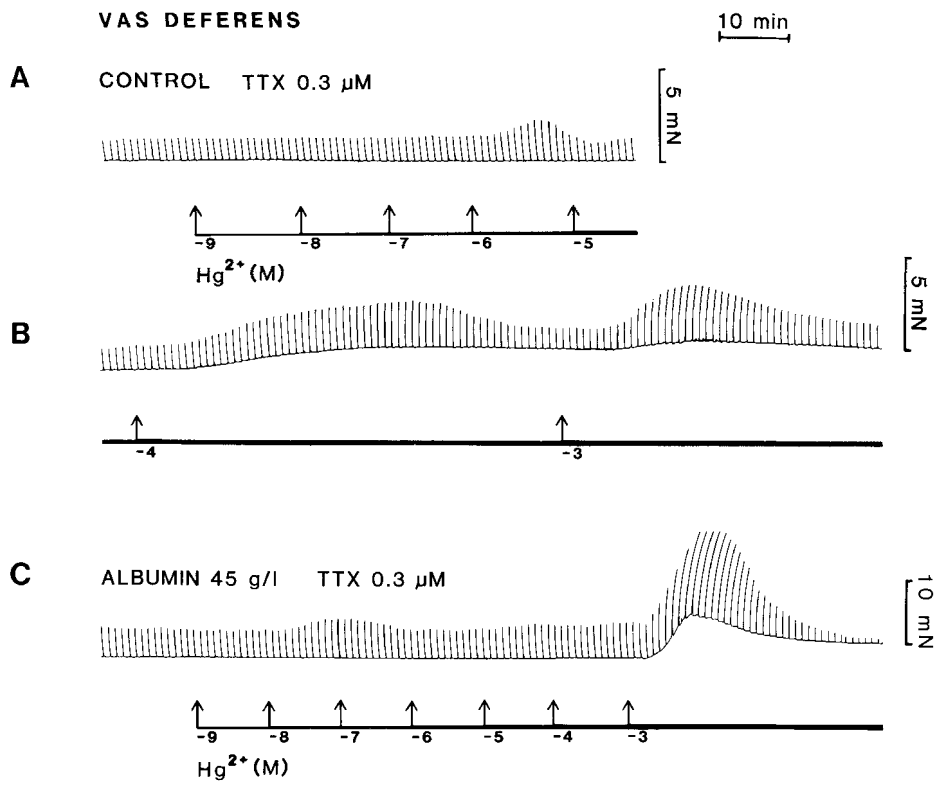

Fig. 2. Hg^{2+} effects in concentrations of 10 logarithms on the direct muscle-stimulated guinea-pig vas deferens. Neurogenic response of stimulation was blocked with 0.3 µm tetrodotoxin (TTX). 2A. Hg^{2+} in 10^{-6} M concentration transiently increased the response to electric stimulation. 2B. Part A continued. Hg^{2+} at 10^{-4} and 10^{-3} M concentration raised the basal tone and coincidently increased the response to electric stimulation. 2C. In the presence of 45 g albumin/l a 10^{-8} M concentration of Hg^{2+} transiently increased the response to electric stimulation. Albumin counteracted the effects of Hg^{2+} at 10^{-4} M concentration, and the concentration of Hg^{2+} had to be increased to raise the basal tone. At 10^{-3} M Hg^{2+} the response to electric stimulation was, after an initial increase, depressed.

10^{-5} M, produced initially strong contractions of the ileum. Relaxation occurred after 10–15 min. Contractions induced by electric stimulation gradually decreased. Hg^{2+}, 10^{-4}–10^{-3} M, after an initial enhancement (3–20 min) depressed the basal tone of the ileum, and the response to electric stimulation faded. These high concentrations (Hg^{2+}, 10^{-4}–10^{-3} M) produced a moderate and transient (30–80 min) contraction of the vas deferens combined with an increase of the response to electric stimulation (Fig. 2B).

Influence of albumin on the effects of Hg^{2+}. Increasing the concentration of $NaHCO_3$ 50% in Tyrode solution did not alter the basal tone or the contractile response of the preparations to direct muscle stimulation. Hg^{2+} had the same effects on the preparations as in ordinary Tyrode solution.

Albumin in itself had no effect on the 'resting tone' or on the contractile response to electric stimulation of the preparations. It blocked the stimulant effect of Hg^{2+}, 10^{-9}–10^{6} M, on the basal tone of the ileum. Contrary to the conditions in ordinary Tyrode solution, Hg^{2+} in the presence of albumin increased the response to direct muscle stimulation in both the ileum and the vas deferens (Fig. 2C).

In the presence of albumin, higher concentrations of Hg^{2+}, 10^{-4}–10^{-3} M, were

needed to increase the basal tone in the ileum than in ordinary Tyrode solution (Fig. 1B). Higher concentrations of Hg^{2+} were also required to decrease the response to direct muscle stimulation. Furthermore, albumin counteracted the depressant effects on basal tone caused by high concentrations of Hg^{2+} (10^{-4}–10^{-3} M) (Fig. 1B).

The stimulant effect on the response to direct muscle stimulation of the vas deferens caused by Hg^{2+} was seen in lower concentrations (10^{-8}–10^{-7} M) and more frequently (10^{-6}–10^{-5} M) in the presence of albumin, especially 4.5 g/l. However, higher concentrations of Hg^{2+}, 10^{-4}–10^{-3} M, depressed the contractile response after a short enhancement (10–15 min). When the high concentration of albumin, 45 g/l, was present, the increase in basal tone was observed at 10^{-3} M of Hg^{2+}.

Response to nerve stimulation

Effects of Hg^{2+}. Hg^{2+} in lower concentrations, 10^{-9}–10^{-7} M, had a stimulant effect on the response to nerve stimulation of the vas deferens (Fig. 3A), and at 10^{-6}–10^{-5} M the stimulant effect was more frequent than on contractions induced by direct muscle stimulation. This was not observed in the ileum (Fig. 4A). High concentrations of Hg^{2+}, 10^{-5}–10^{-3} M, had similar effects on the response of the ileum to nerve stimulation as on the contractions induced by direct muscle stimulation—that is, the depressant effect on the muscle dominated. However, in the vas deferens the increase in response to nerve stimulation was shorter (5–15 min) than that seen on contractions induced by direct muscle stimulation, and in some preparations rhythmic phasic contractions also occurred. The contractions faded in 15–30 min.

Influence of albumin on the effects of Hg^{2+}. A 50% increase of $NaHCO_3$ concentration in Tyrode solution did not influence the response of the preparations to nerve stimulation or the effects of Hg^{2+}. Albumin in itself had no effect on the response to nerve stimulation. In contrast to the experiments with direct smooth-muscle stimulation, Hg^{2+} in concentrations of 10^{-9}–10^{-4} M in the presence of 45 g albumin/l decreased the contractile response of both the ileum and the vas deferens (Fig. 4B, C and 3B).

Discussion

In individuals without subjective symptoms chronic exposure to inorganic mercury at concentrations lower than those considered

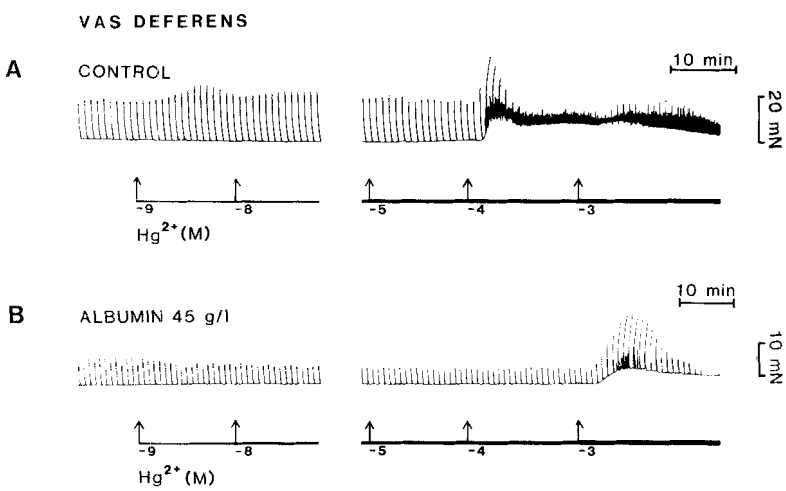

Fig. 3. Effect of Hg^{2+} in concentrations of 10 logarithms on nerve-induced contractions of guinea-pig vas deferens. 3A. At the 10^{-9} M concentration of Hg^{2+} the response to nerve stimulation increased. Hg^{2+} at 10^{-4} M concentration raised the basal tone, and in some preparations rhythmic phasic contractions also appeared. Coincidentally, the response to electric stimulation, after an initial increase, was depressed. 3B. When 45g albumin/l was present, Hg^{2+} in concentrations of 10^{-9}–10^{-4} M successively decreased the contractile response. Concentrations of Hg^{2+} at the 10^{-3} M level raised the basal tone.

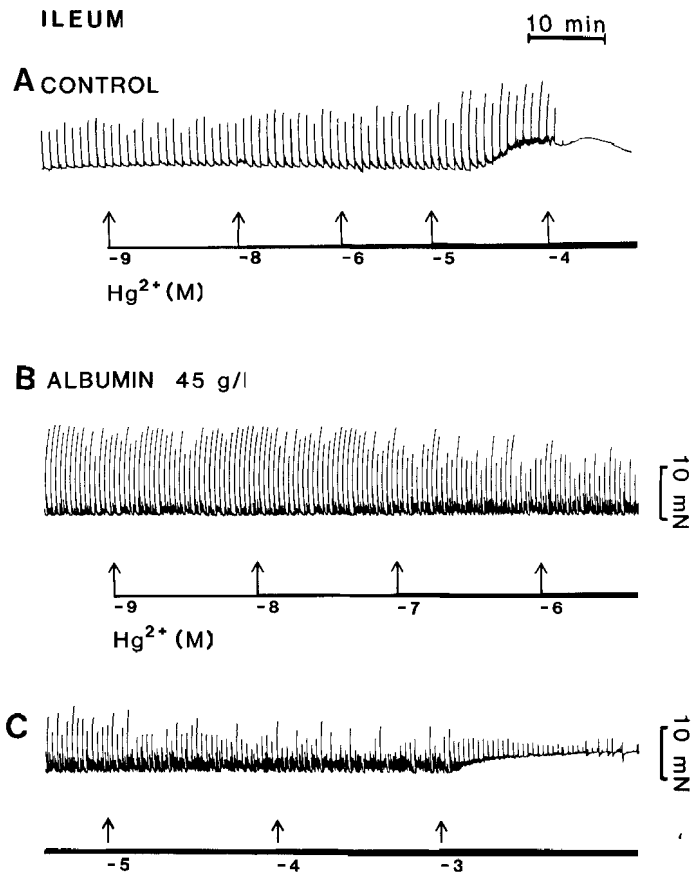

Fig. 4. Effects of Hg^{2+} in concentrations of 10 logarithms on nerve-induced contractions of guinea-pig ileum. 4A. No effect of Hg^{2+} in the 10^{-9}–10^{-6} M range was seen on the contractile response to nerve stimulation. Addition of 10^{-5} M Hg^{2+} increased the basal tone and decreased the contractile response to nerve stimulation. Higher concentrations of Hg^{2+} abolished the response to electric stimulation. 4B. When 45 g albumin/l was present, Hg^{2+} in the 10^{-8}–10^{-6} M range decreased the contractile response to nerve stimulation. 4C. Part B continued. Hg^{2+} in the 10^{-5}–10^{-4} M concentration range further decreased the contractile response to nerve stimulation, and 10^{-3} M raised the tone, whereas the response to electric stimulation faded.

toxic produces effects on both central and peripheral neuronal functions (27–29). Earlier studies have shown that Hg^{2+}, in very low concentrations in in vitro systems, exerts an excitatory action postjunctionally on neuromuscular transmission (22, 30). In the present study the threshold concentration for obtaining an effect of Hg^{2+} was often low. With higher concentrations of Hg^{2+} the fraction of responding preparations increased. The presence of albumin reduces the direct stimulatory effect of Hg^{2+} in concentrations of 10^{-9}–10^{-6} M on the smooth muscle of the ileum. The mechanism of this inhibition is not known, but an interaction between albumin and Hg^{2+} is plausible. Increased accessibility of total calcium in the albumin-containing solutions could have stabilized the muscular membrane. However, this possibility seems less plausible because the concentration of ionized Ca^{2+} was kept constant in the bath. In the vas deferens no effect of Hg^{2+} on the contractile response was seen unless 10^{-4} M was applied. The antagonistic effect of albumin was observed only when 45 g/l was present.

When albumin and Hg^{2+} in concentrations of 10^{-8}–10^{-7} M were present in the bath, contractions induced by direct muscle stimulation were enhanced. Moberg (22) observed a similar effect in a study on ileum in stagnant Tyrode solution without albumin; ileal secretion probably produced high concentrations of proteins in the vicinity of the organ. In the present study the ileal secretion was diluted and dispersed throughout the entire bath system by circulation of the bath solution. This might explain the absence of effect

of low concentrations of Hg^{2+} in Tyrode solution without albumin.

When contractions were induced by nerve stimulation, no effect of Hg^{2+} in the concentration range 10^{-9}–10^{-6} M was seen in the ileum. As Hg^{2+} stimulated the smooth muscle directly, an enhancement would have been noted. Thus, Hg^{2+} seems to inhibit cholinergic neuromuscular transmission. This effect of Hg^{2+} on the ileum was even more pronounced in the presence of 45g albumin/l, and it was also apparent in the vas deferens. It is possible that Hg^{2+} influences the release of transmitters (31–34) and/or inhibits the action of transmitters on the muscle cell membrane (32), which could be consistent with the finding that Hg^{2+} binds to the muscarinic receptor (35).

When albumin was not present in the bath, Hg^{2+} seemed to exert a stimulant effect on the neuromuscular adrenergic transmission of the vas deferens. It is possible that a direct action of Hg^{2+} on the smooth muscle enhanced the motor response to the neurogenic stimulation (36, 37).

In blood serum less than 1% of Hg^{2+} is free or bound to low molecular weight substances (38). In analogy, it is plausible that most of the Hg^{2+} was bound to albumin in our experiment. In the high concentration range (Hg^{2+}, 10^{-5}–10^{-3} M) binding sites on albumin might have been saturated, which would imply incapacity to bind excess of the metal. Then, the concentrations of 'free' or loosely bound Hg^{2+} increases in the solution and augments the probability for the metal to bind to other sites (38).

The levels of mercury in whole blood and interstitial fluid which are caused by mercury from amalgam in man are not known. However, concentrations of Hg in blood plasma, before and after removal of amalgam restorations, have been presented which indicate that the amalgam could be responsible for 2–3 × 10^{-9} M of the Hg (19). The present results imply that Hg^{2+}, even in these low concentrations and also when bound to albumin, may affect neuromuscular transmission. This finding implies that inorganic mercury, even at levels presently considered acceptable, is potentially toxic to neuroeffector systems.

Acknowledgements.—We wish to thank Ms Cecilia Christersson for providing skillful technical assistance. This work was supported by the Faculty of Odontology and the Faculty of Medicine, Karolinska Institute, and the Swedish Dental Society, Stockholm, Sweden.

References

1. Axell T, Nilner K, Nilsson B. Clinical evaluation of patients referred with symptoms related to oral galvanism. Swed Dent J 1983;7:169–78.
2. Haraldson T. Oral galvanism and mandibular dysfunction. Swed Dent J 1985;9:129–33.
3. Hugoson A. Results obtained from patients referred for the investigation of complaints related to oral galvanism. Swed Dent J 1986;10:15–28.
4. Yontchev E, Hedegård B, Carlsson GE. Reported symptoms, diseases, and medication of patients with orofacial discomfort complaints. Int J Oral Maxillofac Surg 1986;15:687–95.
5. Agerberg A. Signs and symptoms of mandibular dysfunction in patients with suspected oral galvanism. Acta Odontol Scand 1987;45:41–8.
6. Molin M, Marklund S, Bergman B, Bergman M, Stenman E. Plasma-selenium, glutathione peroxidase in erythrocytes and mercury in plasma in patients allegedly subject to oral galvanism. Scand J Dent Res 1987;95:328–34.
7. Moberg L-E, Odén A. The microstructure of corroded amalgams. Acta Odontol Scand 1985;43:179–90.
8. Takaku S. Mercury dissolution from dental amalgam into saliva. Bull Tokyo Dent Coll 1985;26:137–52.
9. Hahn LJ, Kloiber R, Vimy MJ, Takahashi Y, Lorscheider FL. Dental 'silver' tooth fillings: a source of mercury exposure revealed by whole-body image scan and tissue analysis. FASEB J 1989;3:2641–6.
10. Hahn LJ, Kloiber R, Leininger RW, Vimy MJ, Lorscheider FL. Whole-body imaging of the distribution of mercury released from dental fillings into monkey tissues. FASEB J 1990;4:3256–60.
11. Svare CW, Peterson LC, Reinhardt JW, et al. The effect of dental amalgams on mercury levels in expired air. J Dent Res 1981;60:1668–71.
12. Abraham JE, Svare CW, Frank CW. The effect of dental amalgam restorations on blood mercury levels. J Dent Res 1984;63:71–3.
13. Vimy MJ, Lorscheider FL. Serial measurements of intra-oral air mercury: estimation of daily dose from dental amalgam. J Dent Res 1985;64:1073–85.
14. Langworth S, Elinder CG, Åkesson A. Mercury exposure from dental fillings. I. Mercury concentrations in blood and urine. Swed Dent J 1988; 12:70–1.
15. Berlin M. Mercury. In: Friberg L, Nordberg GF, Vouk VB, editors. Handbook on the toxicology of metals. Vol II. 2nd ed. Amsterdam: Elsevier/North-Holland Biomedical Press, 1986:387–445.
16. Nilsson B, Nilsson B. Mercury in dental practice.

III. Urinary mercury excretion in dental personnel. Swed Dent J 1986;10:221–32.
17. Olstad ML, Holland RI, Wandel N, Hensten-Pettersen A. Correlation between amalgam restorations and mercury concentrations in urine. J Dent Res 1987;66:1179–82.
18. Langworth S, Köhlbeck KG, Åkesson A. Mercury exposure from dental fillings. II. Release and absorption. Swed Dent J 1988;12:71–2.
19. Molin M, Bergman B, Marklund SL, Schutz A, Skerfving S. Mercury, selenium, and glutatione peroxidase before and after amalgam removal in man. Acta Odontol Scand 1990;48:189–202.
20. Vimy MJ, Takahashi Y, Lorscheider FL, Maternal-fetal distribution of mercury (^{203}Hg) released from dental amalgam fillings. Am J Physiol 1990;258: R939–45.
21. Nylander M, Friberg L, Lind B. Mercury concentrations in the human brain and kidneys in relation to exposure from dental amalgam fillings. Swed Dent J 1987;11:179–87.
22. Moberg L-E. Effects of mercuric ions on isolated guinea-pig ileum. Acta Odontol Scand 1986;44:207–13.
23. Brinster R-L, Cross PC. Effect of copper on the preimplantation mouse embryo. Nature 1972;238: 398–9.
24. Sjöstrand NO. Smooth muscles of vas deferens and other organs in the male reproductive tract. In: Bülbring E, Brading AF, Jones AW, Tomita T, editors. Smooth muscle. London: Edward Arnold Ltd, 1981:367–76.
25. Dahlén S-E. Pulmonary effects of leukotrienes. Acta Physiol Scand 1983; Suppl 512.
26. Moberg L-E, Appelgren B, Sjöstrand NO. Albumin inhibits effects of Cu^{2+} on autonomic postganglionic transmission of guinea-pig ileum and vas deferens. Acta Odontol Scand 1991;49:361–366.
27. Levine SP, Cavender GD, Langolf GD, Albers JW. Elemental mercury exposure: peripheral neurotoxicity. Br J Ind Med 1982;39:136–9.
28. Shapiro IM, Cornblath DR, Sumner AJ, Uzzell B, Spitz LK, Ship II. Neurophysiological and neuropsychological function in mercury-exposed dentists. Lancet 1982;1:1147–50.
29. Lille F, Hazemann P, Garnier R, Dally S. Effects of lead and mercury intoxications on evoked potentials. J Toxicol Clin Toxicol 1988;26:103–16.
30. Aoki T, Oba T, Hotta K. Hg^{2+}-induced contracture in mechanically skinned fibers of frog skeletal muscle. Can J Physiol Pharmacol 1985;63:1070–4.
31. Kostial K, Landeka M. The action of mercury ions on the release of acetylcholine from presynaptic nerve endings. Experientia 1975;31:834–5.
32. Juang MS. An electrophysiological study of the action of methylmercuric chloride and mercuric chloride on the sciatic nerve-Sartorius muscle preparation of the frog. Toxicol Appl Pharmacol 1976; 37:339–48.
33. Miyamoto MD. Hg^{2+} causes neurotoxicity at an intracellular site following entry through Na and Ca channels. Brain Res 1983;267:357–9.
34. Cooper GP, Suszkiw JB, Manalis RS. Heavy metals: effects on synaptic transmission. Neurotoxicology 1984;5:247–66.
35. Burg R von, Northington FK, Shamoo A. Methylmercury inhibition of rat brain muscarinic receptors. Toxicol Appl Pharmacol 1980;53:285–92.
36. Sjöstrand NO. Effects of acetylcholine and some other smooth muscle stimulants on the electrical and mechanical responses of the guinea-pig vas deferens to nerve stimulation. Acta Physiol Scand 1973;89:1–9.
37. Sjöstrand NO. Swedin G. On the mechanism of the enhancement by smooth muscle stimulants of the motor responses of the guinea-pig vas deferens to nerve stimulation. Acta Physiol Scand 1974;90:513–21.
38. Lau S, Sarkar L. Inorganic mercury(II)-binding components in normal human blood serum. J Toxicol Environ Health 1979;5:907–16.

Received for publication 25 February 1991