Theoretical and Practical Considerations on the Problem of Metal–Metal Interaction

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The interaction between two metals, which can be either synergistic or antagonistic, implies that the behavior of one is changed by the presence of the other. Possible mechanisms of these interactions, which include chemical association, competition for carriers, metabolic changes, induction of binding proteins, membrane alterations are discussed.

The interactions between toxic compounds is a loose term which implies that the behavior or effect of one compound is changed by the administration or presence of another. It does not imply that the two compounds interact chemically, although chemical reaction between them or their metabolites is possible. Studies on the interaction between metals are only in an early phase and do not permit the development of generalizations or classifications.

Reaction between Two Metals

The simplest form of interaction between two metals is a chemical reaction, either without or after a chemical transformation, which can be a change in the oxidation state of the metal or, in the case of organometallic compounds, a change in, or cleavage of, the organic radical. If the in vitro conditions do not favor the formation of an insoluble complex between a cationic and anionic species of two metals, such complexes may be formed in vitro due to change in pH or the oxidation state of at least one of the metals. The indication of such a reaction is their increased retention at the injection site when they are administered simultaneously. It is essential, however, that the dose should be low enough to minimize the danger of local tissue damage, as injury can affect absorption (/) without there being any interaction. Thus local tissue injury certainly contributed to the increased retention of selenium at the subcutaneous injection site caused by 28.5–228 μmole/kg Cd²⁺ (2). When 8.0 μmole/kg Cd²⁺ is injected with an equimolar dose of selenite, however, the retention of selenium is increased only slightly and the retention of Cd²⁺ not at all (3), thus refuting the theory of complex formation. When, however, 2.5 μmole/kg Hg²⁺ is administered with an equimolar dose of selenite, the retention of both ions is increased (3). Thus selenite may react with Hg²⁺ but not with Cd²⁺ at the injection site. As mercury selenite precipitates at a lower pH than cadmium selenite (4), the probability of the formation in vivo of the former may be higher than of the latter.

Change in the oxidation state of one metal may favor reaction with another metal, outside the injection site. The conversion of hexavalent selenium to bivalent selenium allows the formation of insoluble metal selenides and, in rats given Hg²⁺ and selenate, black particles that contain mercury and selenium in a 1:1 ratio occur in macrophages and intranuclearly in the renal paroximal tubular cells (5). The formation of mercury selenide supposes not only the reduction of selenate or selenite, but also the concurrent presence of Hg²⁺ to react with selenide. Similar particles have not been found in rats given selenite with Cd²⁺ or tellurate and Hg²⁺ (5), and thus under these circumstances, the heavy metal may not be available when and where selenide is formed.

Selenium and tellurium differently affect the distribution of Hg²⁺. Thus uptake of Hg²⁺ by the liver
is increased by selenium and in the first 24 hr is unaffected by tellurium, whereas uptake by the kidney is decreased by selenium and increased by tellurium (6). The difference in these effects might be explained by the higher reduction potential of SeO₃⁻² compared with that of TeO₃⁻². If the formation of selenide is faster than the formation of teluride and the presence of Hg²⁺ is not a limiting factor, the formation of colloidal particles, which favors deposition in the reticuloendothelial system, must be faster for HgSe than for HgTe.

### Competition for Carriers

Although cations, such as Cd²⁺, Hg²⁺, and Zn²⁺, cannot form complexes with one another, the retention of any of them is increased by the presence of one of the other two (7). A likely explanation for this type of interaction is the depletion of carriers. This would explain why the retention of Hg²⁺ is increased in the absence of any other metal when the dose is increased from 2.5 to 5.0 μmole/kg Hg²⁺ (7).

Many of the metals with known interactions, e.g., Cd²⁺, Hg²⁺, Pb²⁺, Zn²⁺, and selenium, can react with thiol groups. If diffusible thiol compounds contribute to their transport, interaction can be mediated through competition for the same carrier. Thus the retention of Cd²⁺ at the injection site is increased by Hg²⁺ more than the retention of Hg²⁺ by Cd²⁺, and the retention of Zn²⁺ by Hg²⁺ increased more than by Cd²⁺ (7).

Change in the absorption can affect organ distribution. Thus Hg²⁺ decreases the liver content of cadmium by 24% 48 hr after their simultaneous administration, but the difference becomes non-significant when expressed in per cent of the absorbed Cd²⁺ instead of in per cent of the dose (7). Moreover, if the metals are injected intraperitoneally and the Hg²⁺ to Cd²⁺ ratio is increased from 1:1 to 10:1 or more, the liver uptake of Cd²⁺ increases (8).

Zinc and copper which, given in 100–400 times molar excess to Cd²⁺, increase the liver uptake of cadmium at 24 hr. Although at this time Cu²⁺ and Zn²⁺ would have increased the thionein concentration in liver, the possibility cannot be dismissed that the transport of Cd to the liver cells was also influenced. For example, it has been known that the liver uptake of bilirubin or bromsulfophthahalein is facilitated by their binding to albumin in the plasma (9).

Competition for extracellular carrier proteins can contribute to the interactions involving transport and this will depend upon dose and the route of administration. Selenium, which increases the binding affinities of serum proteins for Hg²⁺ (10) also increases the blood concentration and liver uptake of Hg²⁺ (3). However, increase in the blood concentration by selenium is mainly due to its increase in the packed cells (11). The binding of methylmercury to serum proteins is not affected by selenium (10) and selenium decreases the blood concentration of methylmercury with a slight decrease in liver uptake (12).

Interaction may occur on the albumin molecule by competition between Cu²⁺ and Zn²⁺ for common binding sites (13). As, at physiological concentrations and pH, preferential binding of cations to proteins is favored by cooperation between several amino acid residues, interactions may occur not only by competition for the same site, but also by a change in the affinity of one site for a given cation in consequence of the binding of another at a different site.

The mechanism whereby selenium affects the binding of mercury or cadmium is at present not fully understood but, as a first step, selenite must be metabolized in the red blood cells, after which selenium and Hg²⁺ (14), or selenium and Cd²⁺ (15), are bound in a 1:1 ratio to some plasma proteins.

In the intestine, proteins seem to regulate the absorption of some metallic cations. Antagonism by cadmium and zinc of the absorption of copper has been attributed to competition for thionein in the intestinal mucosa (13). It seems now, however, that intestinal Zn²⁺ and Cu²⁺ binding proteins are not identical; while the Zn²⁺ binding protein may be thionein (16), the Cu²⁺ binding protein differs in its amino acid composition from both thionein and chelatin (17). Furthermore, there seems to be an inverse relationship between the synthesis of these binding proteins and cation transfer through the intestine (16, 17). That interaction between metals at the level of intestinal absorption is more complex than competition for a simple carrier is shown by the diversity of conditions which influence the absorption of lead (18, 19).

### Metabolic Interference

Cadmium and Hg²⁺ are able to decrease the formation of dimethyl selenide from selenite (20, 21) because dimethyl selenide formation has an absolute requirement for GSH (22). The reaction of selenite with GSH leads to the formation of seleno-trisulfide (23). Either this compound, or another metabolite of selenite, becomes bound to plasma proteins, mainly β-lipoprotein and globulins (24) and, by an unknown mechanism, promotes the binding of Hg²⁺ and Cd²⁺ to plasma proteins (14, 15). It is not known how the metabolism of selenite,
apart from dimethyl selenide formation, is influenced by Hg²⁺ or Cd²⁺ and what is the essential step in the protective effects between selenium and the two heavy metals, but selenium increases the cleavage of C-Hg bond of phenylmercury (25). Observations that (a) the toxicity of dimethylselenide is increased by Hg²⁺ (20), (b) selenium affects the blood concentration and distribution of metal mercury differently from inorganic mercury (3, 12), (c) in tissue cultures MeHg⁺, on a molar basis is fifty times more efficient against the toxicity of selenite than Hg²⁺ (26); and (d) lead and selenium have a mutual detoxifying effect (27), underline the difficulty in the interpretation of the available biochemical data in relation to the pathological process.

**Induction of Protein Binding Sites**

Thionein is a low molecular weight protein (MW < 10,000) which is able to incorporate or bind a wide variety of metals: Cd²⁺, Cu²⁺, Zn²⁺, Hg²⁺, Ag⁺, Sn²⁺ (28), Co²⁺, and Bi⁺⁺ (29). The most potent inducers of thionein synthesis are Zn²⁺, Cd²⁺, and Hg²⁺ (30–32). Cadmium will replace Zn²⁺, and Hg²⁺ will replace Cd²⁺ in the corresponding metallothionein of the liver (30) and kidneys in vivo (32, 33). Hence interactions between metals that involve thionein can operate through the induction of the protein and through competition for binding on an induced metallothionein. Continuous Cd²⁺ ingestion for example, leads to a considerable increase in the hepatic content of Zn²⁺ and in the renal content of copper bound to thionein (34), while copper and zinc thionein can be isolated from the livers of Cu²⁺ injected rats (35). If uptake by an organ is increased less than the increase in the thionein bound fraction of the metal, toxicity could be decreased. Pretreatment of female rats with low doses of Cd²⁺, however gives a maximum protection against lethal doses of Cd²⁺ 1 and 3 days after pretreatment, though increased thionein content and the capacity to synthesize thionein are maintained for a much longer time (36). Protection given by Cd²⁺ against a renotoxic dose of Hg²⁺ increases the thionein bound Hg²⁺ in the kidneys, but the increase in the total uptake is higher, partly because large molecular proteins bind more Hg²⁺ (33). Thus induction of thionein and the role of thionein in metal induced protection must be carefully analyzed in every instance.

Interaction can produce protection even though the thionein bound fraction of heavy metals is decreased. In the liver, kidneys, and testis, selenium diverts nearly all Hg²⁺ or Cd²⁺ in the soluble fractions from small molecular weight proteins, probably thionein, to larger ones (37, 38).

**Morphological Factors**

Pretreatment with a small but tubulotoxic dose of UO₂²⁺ is able to protect against a subsequent toxic dose. One of the factors in this protection is that the regenerated brush border is more even compared with the normal brush border (39). The brush border is replaced by a smooth membrane after the administration of tubulotoxic doses of HgCl₂ (40), and it is known that in this condition animals can tolerate higher doses of HgCl₂ than otherwise (41). As those metals which are able to initiate a tolerance are also able to develop cross tolerance (41), morphological factors, for example decreased surface area at the part of the tubular cells, where metals are usually taken up, might influence the tubular reabsorption and contribute to their interaction.

**Interaction and Synergistic or Antagonistic Effects**

Interaction between two metals usually results in a decrease in toxicity. If this effect is associated with a shorter half time or lower concentration in the target organ, at least the last link between protection and interaction is established, even though the mechanism of decrease in half time or organ uptake may be unknown. However, the situation usually is more complex; there is a mutual interaction between metals which depend on a chain of reactions; half time and uptake in the target organ are increased, etc.

The purpose of research in this labyrinth is to establish whether the connection between two effects like chemical interaction and protection is coincidental or casual, and to establish the correct sequence of events leading to antagonistic or synergistic effects.

**REFERENCES**

1. Magos, L. Factors affecting the uptake and retention of mercury by kidneys in rats. In: Mercury, Mercurials and Mercaptans. M. W. Miller and T. W. Clarkson, Eds., Charles C Thomas, Springfield, 1973, p. 167.
2. McConnel, K. P., and Carpenter, D. M. Interrelationship between selenium and specific trace elements. Proc. Soc. Exp. Biol. Med. 137: 996 (1971).
3. Magos, L., and Webb, M. Differences in distribution and excretion of selenium and cadmium or mercury after their simultaneous administration subcutaneously in equimolar doses. Arch. Toxicol. 36: 63 (1976).
4. Neville, G. H. J. Selenium. In: Thorpe's Dictionary of Applied Chemistry, Vol. 10, 4th ed. J. F. Thorpe, and M. A. Whiteley, Eds., Longmans, Green & Co., London, 1950, p. 23.
5. Groth, D. H., Stettler, L., and Mackay, G. Interactions of mercury cadmium, tellurium, arsenic and beryllium. In: Effects and Dose–Response Relationships of Toxic Metals. G. F. Nordberg, Ed., Elsevier, Amsterdam, 1976, p. 527.

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6. Eybl, V., Sykora, J., and Mertl, F. Einfluss von Natrium-selenit, Natriumtellurit und Natrium sulfid auf Retention und Verteilung von Quecksilber bei Mausen. Arch. Toxicol. 25: 296 (1969).

7. Magos, L., and Webb, M. The interaction between cadmium, mercury and zinc—administered subcutaneously in a single injection. Arch. Toxicol. 36: 53 (1976).

8. Shank, K. E., and Vetter, R. J. The effects of copper, mercury and zinc on the uptake and distribution of cadmium-115m in the albino rat. Environ. Letters 6: 13 (1974).

9. Serge. G. Kinetics of drugs in the hepatobiliary system. In: Liver and Drugs. F. Orlandi and A.M. Jezequel, Eds., Academic Press, New York, 1972, p. 85.

10. Fynthe, S. C., Chen, R. W., and Fallin, E. Influence of dietary selenite on the binding characteristics of rat serum proteins to mercurial compounds. Chem. Biol. Interact. 15: 51 (1976).

11. Moffit, A. E., Jr., and Clary, J. J. Selenite induced binding of inorganic mercury in blood and other tissues in the rat. Res. Commun. Chem. Pathol. Pharmacol. 7: 593 (1974).

12. Magos, L., and Webb, M. The effect of selenium on the brain uptake of methylmercury. Arch. Toxicol. 37: 201 (1977).

13. Evans, G. W., and Hahn, C. Albumin as a possible site for copper-zinc interaction. In: Trace Element Metabolism in Animals. W. G. Hoekstra et al., Eds., University Park Press, Baltimore, 1974, p. 499.

14. Burk, R. F., et al. Binding of simultaneously administered inorganic selenium and mercury to a rat plasma protein. Proc. Soc. Exp. Biol. Med. 145: 782 (1974).

15. Gasiewicz, T. A., and Smith, J. C. Interactions of cadmium and selenium in rat plasma in vivo and in vitro. Biochim. Biophys. Acta 428: 113 (1976).

16. Richards, M. P., and Cousins, R. J. Mammalian zinc homeostasis: requirements for RNA and metallothionein synthesis. Biochem. Biophys. Res. Commun. 64: 1215 (1975).

17. Evans, G. W., and LeBlanc, F. N. Copper-binding protein in rat intestine: amino acid composition and function. Nutr. Rep. Int. 14: 281 (1976).

18. Task Group on Metal Accumulation. Accumulation of toxic metals with special reference to their absorption, excretion and biological half-times. Environ. Physiol. Biochem. 3: 65 (1973).

19. Klauder, D. S., Murthy, L., and Petering, H. G. Effect of dietary intake of lead acetate on copper metabolism in male rats. In: Trace Substances in Environmental and Animal Physiology. V. D. D. Hemphill, Ed., University of Missouri, Columbia, 1973, p. 131.

20. Parizek, J. Interrelationships among trace elements. In: Effects and Dose-Response Relationships of Toxic Metals. G. F. Nordberg, Ed., Elsevier, Amsterdam, 1976, p. 498.

21. Granthar, H. E., and Baumann, C. A. Selenium metabolism. I. Effects of diet, arsenic and cadmium. J. Nutr. 77: 210 (1962).

22. Granthar, H. E. Enzymic synthesis of dimethyl selenide from sodium selenite in mouse liver extracts. Biochemistry 5: 1089 (1966).

23. Granthar, H. E., and Corcoran, C. Selenotrisulfides. II. Cross linking of reduced pancreatic ribonuclease with selenium. Biochemistry 8: 2557 (1969).

24. Sandholm, M. Function of erythrocytes in attaching selenite-Se onto specific plasma proteins. Acta Pharmacol. Toxicol. 36: 321 (1975).

25. Fang, S. C. Induction of C-Hg cleavage enzymes in rat liver by dietary selenite. Res. Commun. Chem. Pathol. Pharmacol. 9: 579 (1974).

26. Potter, S. D., and Matrone, G. A tissue culture model for mercury-selenium interaction. Toxicol. Appl. Pharmacol. 40: 201 (1977).

27. Rastogi, S. C., Clausen, J., and Srivaskin, K. C. Selenen and lead: mutual detoxifying effects. Toxicology 6: 377 (1976).

28. Sambioni, E., and Marafante, E. Heavy metals in rat liver cadmium binding protein. Environ. Physiol. Biochem. 5: 131 (1975).

29. Piotrowski, J. K., Jadwige, J. K., and Szymanksa, A. Influence of certain metals on the level of metallothionein-like proteins in the liver and kidneys of rats. J. Toxicol. Environ. Health 1: 991 (1976).

30. Webb, M. Binding of cadmium ions by rat liver and kidney. Biochim. Pharmacol. 21: 2751 (1972).

31. Webb, M. Protection by zinc against cadmium toxicity. Biochem. Pharmacol. 21: 2767 (1972).

32. Piotrowski, J. K., Trojanowska, B., and Sapota, A. Binding of cadmium and mercury by metallothionein in the kidneys and liver of rats following repeated administration. Arch. Toxicol. 32: 351 (1974).

33. Webb, M., and Magos, L. Cadmium-thionein and the protection by cadmium against the nephrotoxicity of mercury. Chem. Biol. Interact. 14: 357 (1976).

34. Storard, M. D., and Webb, M. Influence of dietary cadmium on the distribution of the essential metals copper, zinc and iron in tissues of the rat. Chem. Biol. Interact. 15: 349 (1976).

35. Bremer, I., and Young, B. W. Isolation of (copper, zinc)-thioneins from the liver of copper-injected rats. Biochem. J. 157: 517 (1976).

36. Webb, M., and Verschoyle, R. D. An investigation of the role of metallothioneins in protection against the acute toxicity of the cadmium ion. Biochem. Pharmacol. 25: 673 (1976).

37. Chen, R. W., Whanger, P. D., and Fang, S. C. Diversion of mercury binding in rat tissues by selenium: a possible mechanism of protection. Pharmacol. Res. Commun. 6: 571 (1974).

38. Chen, R. W., Whanger, P. D., and Weswig, P. J. Selenium-induced redistribution of cadmium binding to tissue proteins: a possible mechanism of protection against cadmium toxicity. Bioinorg. Chem. 4: 125 (1975).

39. Haven, F. Tolerance to uranium compounds. In: Pharmacology and Toxicology of Uranium Compounds. C. Vogtlin and H. C. Hodge, Eds., McGraw-Hill, New York, 1949, p. 729.

40. Price, R. G., and Kempson, S. A. The effect of mercuric chloride on rat kidney cortical plasma membranes. Biochem. Soc. Trans. 3: 294 (1975).

41. Yoshiwaka, H. Preventive effect of pretreatment with low dose of metals on the acute toxicity of metals in mice. Ind. Health (Japan) 8: 184 (1970).
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