1. Lyon, M. W., *Proc. U.S. Nat. Hist. Mus.*, 1903, 20, 425.
2. Ramaswami, L. S., *J. Mys. Univ.*, 1933, 7, 1.
3. Wimsatt, W. A., *J. Mammal.*, 1945, 26, 23.
4. Gopalakrishna, A., *Proc. Ind. Acad. Sci.*, 1947, 26, 219.
5. Uchida, T., *Sci. Bull. Fac. Agr., Kyushu Univ.*, 1950, 12, 11.
6. McKean, J. L. and Hamilton-Smith, E., *Victoria Natur.*, 1967, 84, 203.
7. Madhavan, A., *Mammalia*, 1971, 35, 283.
8. Gopalakrishna, A., Thakur, R. S. and Madhavan, A., *Dr. B. S. Chauhan Comm. Vol.*, 1975, p. 225.
9. Wimsatt, W. A. and Trapido, H. T., *Amer. J. Anat.*, 1952, 91, 415.
10. Gopalakrishna, A., *Curr. Sci.*, 1954, 23, 60.
11. —, *Proc. Nat. Inst. Sci. India*, 1955, 21, 29.
12. —, *Curr. Sci.*, 1964, 33, 558.
13. — and Choudhari, P. N., *J. Bombay Nat. Hist. Soc.*, 1977, 74, 1.
14. Matthews, L. H., *Trans. Zool. Soc. London*, 1937, 23, 224.
15. —, *Proc. Zool. Soc. London*, 1941, 111, 289.
16. Gopalakrishna, A., *Proc. Nat. Inst. Sci. India*, 1950, 16, 93.
17. Ramakrishna, P. A., *J. Mys. Univ.*, 1951, 12, 107.
18. Ramaswamy, K. R., *Proc. Nat. Inst. Sci. India*, 1961, 27, 287.
19. Ramaswami, L. S. and Anandkumar, T. C., *Experientia*, 1963, 19, 321.
20. Gopalakrishna, A., Khaparde, M. S. and Sapkal, V. M., *J. Bombay Nat. Hist. Soc.*, (In press).
21. Madhavan, A., Patil, D. R. and Gopalakrishna, A., *Ibid.*, (In press).
22. Gopalakrishna, A. and Madhavan, A., *Ibid.*, (In press).
23. Brosset, A., *Ibid.*, 1962, 59, 707.
24. Gopalakrishna, A. and Rao, K. V. B., *Ibid.*, (In press).
25. Ramakrishna, P. A. and Rao, K. V. B., *Curr. Sci.*, 1977, 46, 270.
26. Gopalakrishna, A. and Phansalkar, R. B., *Ibid*, 1970, 39, 309.
27. Karim, K. B., *Ibid.*, 1973, 42, 823.
28. Moghe, M. A., *Bull. Zool. Soc. Coll. Sci. Nagpur*, 1960, 2, 1.
29. Bradshaw, G. V. R., *Science, N.Y.*, 1962, 135, 645.
30. Barlow, J. and Tamsitt, J. R., *Can. J. Zool.*, 1968, 46, 290.
31. Davis, W. B., *J. Mammal.*, 1970, 51, 105.
32. Burns, R. J., *Ibid.*, 1970, 51, 391.
33. Medway, Lord, *J. Zool.*, 1971, 165, 261.
34. Marshall, A. J., *J. Endocrinol.*, 1953, 9, 42.
35. Gopalakrishna, A. and Murthy, K. V. R., *Bull. Zool. Soc. Coll. Sci. Nagpur*, 1960, 2, 19.
36. — and Karim, K. B., *Curr. Sci.*, 1971, 40, 490.

COBALT TOXICITY AND ITS REVERSAL BY IRON AND MAGNESIUM IN *OGAWA* SEROTYPES OF *VIBRIO CHOLERAE* AND *VIBRIO ELTOR*

C. N. NAGESHA, B. P. LALITHAMMA, S. NARASIMHA RAO, N. C. ANANTHAKRISHNA AND I. KARUNA SAGAR

Departments of Microbiology and Biochemistry, Kasturba Medical College, Mangalore 575 001, *India*

ABSTRACT

Toxic effects of Co^{2+} on growth, glucose utilisation and acid production were studied, and the capability of Fe^{3+} and Mg^{2+} for counteracting Co^{2+} toxicity was investigated in *Ogawa* serotypes of *Vibrio cholerae* and *Vibrio eltor*. Bivalent Co induced 50% growth inhibition in *V. cholerae Ogawa* and *V. eltor Ogawa* at 150 μg and 100 μg per 10 ml test medium respectively. It was toxic to both the biotypes and destroyed their metabolic activity at 250 μg. Co^{2+} toxicity was reversed by both Fe^{3+} and Mg^{2+} to a varying degree in *V. eltor Ogawa*. In *V. cholerae Ogawa*, Mg^{2+} reversed Co^{2+} effects to a considerable extent while Fe^{3+} supplementation caused the total extinction of all the metabolic parameters in Co^{2+} toxicosed cells. The results indicate interesting interactions between Co^{2+}, Mg^{2+} and Fe^{3+} in these strains.

INTRODUCTION

IN compound and ionic form, elements such as H, Na, K, Mg, Ca, Fe, C, N, P, O and S are constituents of many types of living cells, whereas several other elements occur in smaller amounts or as trace elements[1]. Metals of low atomic weights are less toxic than those of high atomic weight; moreover, bivalent metals are more toxic than monovalent metals[2].

Extra physiological concentrations of certain heavy metals are known to produce pathological changes in animals, plants and micro-organisms. The toxic effects of different concentrations of Co, Zn, Ni and Mo in mostly non-pathogenic organisms have been demonstrated. Responses towards certain pairs of metals varied and the interaction occurring in one microbial species need not occur in others. In *Neurospora crassa* deranged Fe and Mg metabolism particularly caused

by Co was observed[3]. In *N. crassa* an Fe-binding compound has been isolated from the culture fluid of organisms grown under conditions of Co-toxicity[4]. Further, increasing concentrations of Co in the medium resulted in the increased production of an Fe-binding compound and a corresponding fall in the catalase activity of *N. crassa*[5]. Co-toxicity was correlated with Fe deficiency in *Aspergillus niger*[6] and in *Micrococcus pyogenes* var. *aureus*[7], Fe was more effective than Mg in the alleviation of Co-toxicity in these organisms. Cobalt-inhibited growth of *A. niger* was accompanied by decreased glucose utilisation and acid production and under these conditions the metabolism of several organic acid intermediates of glucose breakdown was affected[8].

The current report is concerned with the study of interrelationships between Co^{2+} toxicity and its reversal by Fe^{3+} and Mg^{2+} in *Ogawa* serotypes of pathogenic vibrios.

Materials and Methods

V. cholerae neotype strain, *Ogawa* serotype (NCTC 8021) and *V. cholerae* biotype *eltor*, *Ogawa* serotype (NCTC 10255) obtained from Central Public Health Laboratory, London, were used.

Cobalt chloride (BDH), ferric ammonium citrate (Merck) and magnesium sulphate (BDH) were of Analar grade. Ferric ammonium citrate was used in the present study since ferrous salts are easily oxidised to ferric form. Sterile glass-distilled water was employed in preparing metal solutions separately so that 100 ml of each solution contained 100 mg of the metal. The final concentrations were expressed as μg of metal per tube containing 10 ml test medium.

Each 100 ml lot of test medium contained peptone (1 g), sodium chloride (0·5 g), and glucose (1 g). Each 100 ml lot of peptone water contained only peptone, (1 g), and sodium chloride (0·5 g). The pH of these culture media was adjusted to 7·6.

Cobalt solution was added aseptically to the tubes containing 10 ml test medium to provide the metal concentrations ranging from 50–1000 μg/tube. Experiments conducted to determine toxicity reversals contained Co^{2+} sufficient to induce 50% growth inhibition and reversing metals, Fe^{3+}/Mg^{2+} in concentrations from 50–1000 μg per tube. Aliquots of 0·05 ml of 20 h peptone water culture of vibrios containing approximately 10^8 viable cells were used to inoculate the tubes. After incubation for 24 h, the growth was measured colorimetrically at 660 nm. Glucose was estimated by the technique of Follin and Wu[9]. Acid production was determined by titrating 2 ml aliquots of culture medium against 0·005 N NaOH using bromothymol blue as indicator. All experiments were repeated 5 times and the average values were taken.

Results

Co^{2+} effects on growth.—Bivalent Co was toxic to both the biotypes (Table I). It induced 50% growth inhibition in *V. cholerae Ogawa* and *V. eltor Ogawa* at 150 μg and 100 μg respectively. While the growth of these pathogenic vibrios was almost at par with 50 μg, it reached extinction level at 250 μg in both the biotypes. At 200 μg, the growth was inhibited by 90% in *V. cholerae* and 68% in *V. eltor*. Co^{2+} thus appears less tolerated by *V. cholerae Ogawa* than by *V. eltor Ogawa* beyond 150 μg although the tolerance of the former to Co^{2+} at 100 μg was greater than that of the latter, the growth values being 70% and 50% respectively.

Co^{2+} effects on glucose utilisation and acid production.—At concentration of Co^{2+} inducing 50% growth inhibition, glucose utilisation and acid production values reached 50% and 45% of maximals respectively in *V. cholerae Ogawa*, and 65% and 75% in *V. eltor Ogawa* (Table I). Interestingly, at 200 μg, *V. cholerae Ogawa* achieving a growth of only 10% removed 33% of the initial supply of glucose without any acid production. This suggests the possible suppression of catabolic activity at this metal concentration, although glucose was imbibed by the Co^{2+} treated cells. In contrast, in *V. eltor Ogawa* yielding a growth of 32% at 200 μg, the utilisation and production values were 48% and 50% respectively. Further, at and above 200 μg, Co^{2+} totally destroyed the metabolic activity in both the biotypes as evident from the values of all the responses reaching extinction levels.

Effects of Fe^{3+} and Mg^{2+} on Co^{2+} toxicity.—In *V. cholerae Ogawa*, Fe^{3}, besides its absolute failure to alleviate Co^{2} toxicity, also caused total extinction of all metabolic responses, while Mg^{2} reversed Co^{2} influences to a considerable degree (Table II). On the other hand, both Fe^{3} and Mg^{2+} reversed Co^{2+} effects in *V. eltor Ogawa* to a varying extent (Table III).

It is evident from Table II that supplementation of Fe^{3+} from 50 to 1000 μg did not restore Co^{2} inhibited bacterial growth, glucose utilisation and acid production to any degree in *V. cholerae Ogawa*. Interestingly, Fe^{3} supplementation resulted in the destruction of metabolic functions as revealed by the values of all the responses reaching extinction levels. Experiments to determine the effects of Fe^{3+} alone on these vibrio types revealed 100% growth, glucose utilisation and acid production.

TABLE I

Cobalt^{2+} effects on metabolic parameters in Ogawa *serotype of* V. cholerae *and* V. eltor*

	Co^{2+} in μg/10 ml medium						
	0	50	100	150	200	250	300–1,000
	Ogawa serotype of *V. cholerae*						
Growth	100	74	70	50	10	0	0
Glucose utilisation	100	100	75	50	33	0	0
Acid production	100	84	73	45	0	0	0
	Ogawa serotype of *V. eltor*						
Growth	100	72	50	42	32	0	0
Glucose utilisation	100	82	65	62	48	0	0
Acid production	100	81	75	68	50	0	0

* Values expressed as percentage of control.

TABLE II

Influences of Fe^{3+} and Mg^{2+} supplementation on Co^{2+} effects in Ogawa *serotype of* V. cholerae*

Analysis	Fe^{3+}/Mg^{2+} in μg/10 ml medium														
	c_1	c_2	50	100	150	200	250	300	400	500	600	700	800	900	1000
	$Co^{2+} + Fe^{3+}$														
Growth	100	50	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose utilisation	100	50	0	0	0	0	0	0	0	0	0	0	0	0	0
Acid production	100	45	0	0	0	0	0	0	0	0	0	0	0	0	0
	$Co^{2+} + Mg^{2+}$														
Growth	100	50	54	65	72	72	72	72	72	72	72	72	72	72	72
Glucose utilisation	100	50	80	80	100	100	100	100	100	100	100	100	100	100	100
Acid production	100	45	60	60	65	70	70	74	74	70	70	64	60	60	60

* Values expressed as percentage of control.
c_1 Control without any metal.
c_2 Control with Co^{2+} (150 μg) inducing 50% growth inhibition.

In contrast, 150 μg of Mg^{2} restored bacterial growth of 72% with 100% glucose utilisation. Increasing concentrations of this metal above the maxima did not improve the growth pattern which remained at the same level and the situation of glucose utilisation also unchanged remaining 100%. Supplementation with 50–400 μg of Mg^{2+} restored acid production from 60% to 74% with a gradual slight fall at higher concentrations.

Table III shows that in *V. eltor Ogawa,* 200 μg of Mg^{2} restored maximal growth of 85%. Addition of 50–500 μg of Fe^{3+} caused only a slight improvement in growth (10%) and acid production (6%), while glucose utilisation level remained the same. Interestingly, higher concentrations of Fe^{3+} were inhibitory to all the three parameters tested. On the other hand, supplementation of 50–1000 μg of Mg^{2+} restored bacterial growth from 50% to 85%, glucose utilisation from 65% to 87% and acid production from 75% to 81%.

DISCUSSION

From the data presented here, Mg^{2+} is more effective than Fe^{3} in the alleviation of the

TABLE III

Influences of Fe^{3+} and Mg^{2+} supplementation on Co^{2+} effects in Ogawa *serotype of* V. eltor*

Analysis	Fe^{3+}/Mg^{2+} in μg/10 ml medium														
	c_1	c_2	50	100	150	200	250	300	400	500	600	700	800	900	1000
							$Co^{2+} + Fe^{3+}$								
Growth	100	50	55	60	60	60	60	60	60	60	50	45	34	34	34
Glucose utilisation	100	65	65	65	65	65	65	65	65	65	60	52	52	52	52
Acid production	100	75	79	80	81	81	81	81	81	81	79	75	66	66	66
							$Co^{2+} + Mg^{2+}$								
Growth	100	50	66	76	81	85	85	85	85	85	85	85	85	85	85
Glucose utilisation	100	65	65	65	87	87	87	87	87	87	87	87	87	87	87
Acid production	100	75	75	77	81	81	81	81	81	81	81	81	81	81	81

* Values expressed as percentage of control.

c_1 Control without any metal.

c_2 Control with Co^{2+} (100 μg) inducing 50% growth inhibition.

deleterious effects of Co^{2+} in *V. eltor Ogawa*. While beneficial influences are seen with Mg^{2+} in *V. cholerae Ogawa*, Fe^{3} not only has failed in ameliorating Co^{2+} effects but also caused absolute toxicity resulting in total extinction of all metabolic responses. The salient differences noticed in the specific reversal pattern of Co^{2+} toxicity are contrary to the observations reported for *M. pyogenes* var. *aureus*[7] and by Adiga *et al.*[6], for *A. niger* where Fe^{3+} was more effective than Mg^{2+} in reversing Co^{2+} toxicity.

Further, Fe^{3+} alone is not toxic to *V. cholerae Ogawa*. This indicates that Fe^{3} as such has no adverse effects on this vibrio to any extent, while it is drastically toxic in combination with Co^{2+}. On the other hand, it is interesting to recall our previous observation that although Fe^{3+} did not enhance Ni^{2+} toxicity, it totally failed to alleviate this effect in *V. cholerae Ogawa*[10]. The exact mechanisms in which Fe^{3+} functions as totally toxic to Co^{2+} treated cells of *V. cholerae Ogawa* in their metabolism remains to be investigated. This observation may indicate biochemical differences between the two biotypes, and more detailed enzyme studies may reveal the intricate mechanism involved in their metabolic functions.

The difference observed in metabolic responses when the cells are exposed to Co^{2+} at 200 μg in *V. cholerae Ogawa* and *V. eltor Ogawa* appear to be significant since it was consistent. Also the reversal findings with Fe^{3+} indicate marked differences in the pattern of behaviour of the two strains tested.

ACKNOWLEDGEMENT

We thank Dr. S. Lapage, Curator, Central Public Health Laboratory, London, for kindly supplying the bacterial strains.

1. Frobisher, M., *Fundamentals of Microbiology*, 8th ed., W. B. Saunders Co. (Philadelphia), 1968, p. 70.
2. Wilson, G. S. and Miles, A. A., *Topley and Wilson's Principles of Bacteriology and Immunity*, Edward Arnolds Ltd. (London), 1964, p. 141.
3. Sivarama Sastry, K., Adiga, P. R., Venkatasubramanyam, V. and Sarma, P. S., *Biochem. J.*, 1962, 85, 486.
4. Padmanabhan, G. and Sarma, P. S., *Ibid.*, 1964, 98, 330.
5. — and —, *Arch. Biochem. Biophys.*, 1965, 111, 147.
6. Adiga, P. R., Sivarama Sastry, K., Venkatasubramanyam, V. and Sarma, P. S., *Biochem. J.*, 1961, 81, 545.
7. Narasimha Rao, S. and Nagesha, C. N., *Indian J. Expt. Biol.*, 1967, 5, 182.
8. Sivarama Sastry, K., Adiga, P. R., Padmanabhan, G. and Sarma, P. S., *Arch. Biochem. Biophys.*, 1962, 99, 351.
9. Folin, O. and Wu, H., *J. Biol. Chem.*, 1920, 41, 367.
10. Lalithamma, B. P., Karuna Sagar, I., Narasimha Rao, S. and Nagesha, C. N., *Curr. Sci.*, 1976, 45, 578.