TABLE I

Deformability Index Values of Graphite in the As-Cast and Ferritised S.G. Irons

Treatment	% deformation	Deformability index
As-cast	6	2·8
As-cast	13	2·0
As-cast	25	1·0
Ferritised	8	2·3
Ferritised	15	1·5
Ferritised	23	1·0

The ductile behaviour of the S.G. iron can therefore be attributed to the ease with which the graphite nodules change shape on deformation. This is not possible in the gray cast irons, since the graphite flakes act as stress raisers and do not deform with the metal.

CONCLUSION

The graphite nodules of S G. iron deform along with the matrix and the nodular shape changes to ellipsoidal shape on deformation. The calculated deformability index values show that the relative magnitude of deformation of graphite with respect to the matrix changes with the degree of strain.

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ROLE OF MAGNESIUM AND IRON IN THE REVERSAL OF NICKEL TOXICITY IN *OGAWA* SEROTYPES OF *VIBRIO CHOLERAE* AND *VIBRIO ELTOR*

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ABSTRACT

The effect of different concentrations of Ni^{2+} on growth, acid production and glucose utilisation of *Ogawa* serotypes of *Vibrio cholerae* and *Vibrio eltor* has been studied. Ni^{2+} was toxic to both, particularly for acid production. The reversibility of Ni^{2+} toxicity by Fe^{3+} and Mg^{2+} showed that Ni^{2+} induces a conditioned Mg^{2+} deficiency in both *V. cholerae* and *V. eltor*.

INTRODUCTION

THOUGH nickel has been placed with "unfamiliar trace elements" from the point of view of microbial nutrition[1], evidence as to its essential function is gradually accumulating[2]. For instance, Abelson and Aldous[3] demonstrated that excess of nickel and other heavy metals interfere with magnesium metabolism in microorganisms and Healy *et al*[4] observed that nickel reduced succinic dehydrogenase but doubled catalase activity in *Neurospora crassa*. Interestingly, whereas magnesium was effective in counteracting nickel toxicity in *Aspergillus niger*[5] and *Neurospora crassa*[6], zinc was effective in *Chlorella vulgaris*[7] and both iron and magnesium proved effective in reversing nickel toxicity in *Micrococcus pyogenes* var. *aureus*[8].

In *Inaba* serotypes of *Vibrio cholerae and Vibrio eltor*, Karuna Sagar *et al*[9] noted that whereas iron was more effective in counteracting nickel toxicity in the former, magnesium was in the latter. In this communication we present the results of our experiments with *Ogawa* serotypes of *Vibrio cholerae* an *Vibrio eltor*.

MATERIALS AND METHODS

One strain of *Vibrio cholerae Ogawa* (NCTC 8021) and one strain of *Vibrio eltor Ogawa* (NCTC 10255) obtained from Central Public Health Laboratory, London, were used.

The test medium of pH 7·6 contained tryptone (Oxoid) 1%, sodium chloride (BDH) 0·5% and glucose (BDH) 1%. Solutions of nickel sulphate (May and Baker Ltd.), ferric ammonium citrate (Merck) and magnesium sulphate (Merck) were prepared to contain 1000 mcg of metal per ml.

Ni^{2} solution was added aseptically to the tubes containing 10 ml test medium to provide the metal concentrations ranging from 50 to 1000 mcg per tube Experiments conducted to determine toxicity reversals contained Ni^{2+} sufficient to induce 50% growth inhibition and reversing metals, Fe^{3+} / Mg^{2+} in concentrations from 50 to 1000 mcg 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, growth was measured colorimetrically at 660 nm. Glucose utilisation was determined by following its disappearance from the medium by the technique of Folin and Wu[10]. Acid production was estimated by titrating 2 ml aliquots of culture medium against 0·005 N NaOH using bromothymol blue as indicator.

All the experiments were repeated four times and the results were consistent. Average values were expressed as percentage of values recorded for control culture.

Results

Table I shows the effect of Ni^{2+} on *Ogawa* serotypes of *Vibrio cholerae* and *Vibrio eltor*. Complete inhibition of growth occurred at 600 mcg in the former and 500 mcg in the latter. However, 50% growth inhibition was noted at 200 mcg level in both the biotypes. The fall in acid production was more in *V. cholerae* than in *V. eltor*. Thus at 200 mcg which produced 50% growth inhibition in both biotypes, acid production was seen to the extent of 25% in *V. cholerae* and 44% in *V. eltor*.

Table II shows the effect of Fe^{3+} and Mg^{2+} supplementation to *V. cholerae* culture containing a concentration

TABLE I

Effect of Ni^{2+} on Ogawa *serotypes of* V. cholerae *and* V. eltor (*Values expressed as percentage of control*)

	Concentration of Ni^{2+} (mcg/10 ml medium)									
	Nil	50	100	150	200	250	300	400	500	600
					V. cholerae					
Growth ..	100	83	65	58	50	44	44	44	39	..
Acid production ..	100	50	50	30	25	12	12	12	12	..
Glucose utilisation ..	100	100	80	70	70	50	50	50	50	..
					V. elter					
Growth ..	100	90	70	55	50	35	25	20	..	..
Acid production ..	100	62	60	44	44	20	12	12	..	..
Glucose utilisation ..	100	80	80	80	60	60	40	40	..	..

TABLE II

Effect of Mg^{2+} and Fe^{3+} in presence of 200 *mcg of Ni^{2+}* (50% *growth inhibition*) *in* V. cholerae Ogawa (*values expressed as percentage of control*)

		Concentration of Mg^{2+} / Fe^{3+} (mcg/10 ml medium)												
	Control	Nil	50	100	150	200	250	300	400	500	600	700	800	900	1000
						Ni (200 mcg) + Mg									
Growth	100	50	58	70	80	80	80	80	80	80	80	89	95	95	95
Acid production	100	25	40	45	50	50	55	55	65	75	75	75	75	75	75
Glucose utilisation	100	70	70	70	80	100	100	100	100	100	100	100	100	100	100
						Ni (200 mcg) + Fe									
Growth	100	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Acid production	100	25	25	25	5	25	25	25	25	25	25	25	25	25	25
Glucose utilisation	100	70	70	70	70	70	70	70	70	70	70	70	70	70	70

TABLE III

Effect of Mg^{2+} and Fe^{3+} supplementation on Ni^{2+} inducing 50% growth inhibition in V. eltor Ogawa
(values expressed as percentage of control)

	Con-trol	Nil	Concentration of Mg^{2+} / Fe^{3+} (mcg/10 ml medium) 50	100	150	200	250	300	400	500	600	700	800	900	1000
						Ni (200 mcg) + Mg									
Growth	100	50	68	68	68	70	78	78	84	84	84	84	84	84	84
Acid production	100	44	50	50	50	50	50	50	50	60	60	60	60	60	60
Glucose utilisation	100	60	60	60	60	80	100	100	100	100	100	100	100	100	100
						Ni (200 mcg) + Fe									
Growth	100	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Acid production	100	41	40	40	32	22	22	22	22	18	18	18	18	18	18
Glucose utilisation	100	60	60	60	60	55	55	50	50	50	50	50	50	50	50

of Ni^{2+} (200 mcg) inducing ha f maximal growth. Mg^{2+} was partially effective in reversing Ni^{2+} toxicity. Growth reached a maximum value of 95% at 800 mcg level. Though normal utilisation of glucose was observable at 200 mcg, no increase in acid production occurred beyond 75%. Fe^{3+} was completely ineffective in reversing Ni^{2+} toxicity.

A similar situation was evidenced in the case of *V. eltor* also (Table III). Supplementation of Mg^{2+} brought about a maximum of 84% in growth at 400 mcg. Glucose utilisation reached the control value at 250 mcg but optimal acid production was only 60% (500 mcg). Fe^{3+} was not only ineffective in reversing Ni^{2+} toxicity, but was in fact inhibitory, particularly to acid production.

DISCUSSION

The above results clearly show that Ni^{2+} was toxic to both the biotypes of vibrios. Though half maximal growth was attained at the same concentration of Ni^{2+} in both the biotypes, a greater inhibition of acid production was noted in *V. cholerae*.

Mg^{2+} was partially effective in reversing the toxicity due to Ni^{2+} in both the biotypes. Though glucose utilisation was restored in both *V. cholerae* and *V. eltor* acid production was still low. Abelson and Aldous[3] indicated that the primary influence of Mg^{2+} is a control on the toxic metal uptake which presumably results in the depression of intracellular concentration of toxic ions to non-toxic levels. If their surmise is right it would be natural to expect all adversee influence of metal toxicities to be simultaneously annulled. Since this does not happen in Ni^{2+} toxicity control of ion uptake is not perhaps the only mechanism of reversal of metal toxicity by Mg^{2+}. In fact, Adiga *et al*[5] and Narasimha Rao and Nagesha[8] arrived at similar conclusions from their studies on toxicity reversals.

Fe^{3+} totally failed to alleviate Ni^{2+} toxicity. This is interesting from the viewpoint of the observations of Narasimha Rao and Nagesha[2] and that of Adiga *et al*[5] that both Fe^{3+} and Mg^{2+} were effective in reversing Ni^{2+} toxicity. Our results indicate that Ni^{2+} might be inducing a conditioned deficiency of Mg^{2+} in *Ogawa* serotypes of *V. cholerae* and *V. eltor*.

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