Interrelationships in Trace Element Metabolism in Metal Toxicities in *Corcyra cephalonica* St.* (27133)

P. R. ADIGA, K. SIVARAMA SASTRY, V. VENKATASUBRAMANYAM† AND P. S. SARMA
Department of Biochemistry, Indian Institute of Science, Bangalore, India

It is becoming clear that heavy metal toxicities can affect iron or magnesium metabolism in various organisms. In plants, chlorosis caused by several heavy metals can be corrected by iron salts(1). There is also evidence that in plants as well as in some microorganisms parallel phenomena occur(2,3,4), though only recently it has been demonstrated that the influence of iron and magnesium is characteristically different(5,6). Such interrelationships have not been studied as extensively in other species; however, in rats, zinc toxicity is known to result in deranged iron metabolism(7,8), though the growth inhibition in this case is not reversed by additional iron(9). In *Corcyra cephalonica* St., an organism with nutritional requirements similar to rats(10,11), the observed effects of zinc toxicity resemble corresponding manifestations in rats(12,13). In view of this, the influence of iron and magnesium on cobalt, nickel and zinc toxicities has been studied in *Corcyra* larvae.

Methods. The technic employed in rearing *Corcyra* larvae, preparation of diets and in determining growth has been described (12). In all cases normal 10-15-day-old larvae were transferred in lots of 30 to 10 g of appropriate diets, consisting of wheat flour supplemented with metal salts as shown in tables below. Growth was followed for 3 weeks thereafter. Controls on basal diet of wheat flour alone were always maintained, since the final weights of larvae were dependent upon their initial weights as well as on environmental conditions of temperature and humidity. Metal salts (analytical grade, Merck & Co., Ltd.) used were $ZnSO_4 \cdot 7H_2O$, $CoCl_2 \cdot 6H_2O$, $NiSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$ and ferric ammonium citrate; all included to provide the required amounts of metals per 10 g of diet. All experiments were repeated at least 4 times.

* Supported financially by the Council of Scientific & Industrial Research, India.

† Present address: A. M. Jain College, Meenambakkan, Madras.

TABLE I. Influence of Increasing Levels of Co on Growth of *Corcyra* Larvae.

Co level (mg/10 g diet)	Start	Weeks of exp. 1	2	3	Wt gain at end of exp.
	Wt of 10 larvae (mg)				
Nil (control)	6.3	32.5	130.8	263.0	256.7
1.0	5.5	26.6	96.7	209.3	203.8
1.5	6.1	25.0	75.7	183.3	177.2
2.0	5.2	16.2	44.4	78.0	72.8
2.5	5.7	14.5	41.1	75.1	69.4
3.0	5.2	14.2	40.2	65.2	60.0

Results. In Tables I and II are presented data on the effects of increasing levels of Co and Ni on growth of *Corcyra* larvae. Levels beyond the highest shown herein proved lethal, hence the corresponding values have not been included. The concentrations needed to produce about 60% inhibition of growth by the end of the experimental period are close to 2 mg for both Co and Ni. By comparison with the data of Sivarama Sastry *et al.*(12), it would appear that Co and Ni are more toxic to *Corcyra* than Zn, which has to be administered at 9 mg level for similar results.

In all subsequent studies, the influence of Fe and Mg has been studied using these concentrations of toxic metals, *viz.*, 2 mg Co, 2 mg Ni and 9 mg Zn. Fe and Mg were supplemented as shown in Tables III, IV and V below, along with toxic metals. These levels were optimal as found by a few preliminary

TABLE II. Influence of Increasing Levels of Ni on Growth of *Corcyra* Larvae.

Ni level (mg/10 g diet)	Start	Weeks of exp. 1	2	3	Wt gain at end of exp.
	Wt of 10 larvae (mg)				
Nil (control)	5.7	32.5	114.2	250.0	244.3
1.0	4.8	20.2	82.7	178.0	173.2
1.5	5.4	19.6	65.8	159.0	153.6
2.0	5.2	18.4	55.2	79.8	74.6
2.5	5.7	16.0	48.1	60.5	54.8
3.0	4.4	14.0	42.1	58.2	53.8

TABLE III. Influence of Fe and Mg on Co Toxicity in *Corcyra* Larvae.

Supplements to diet* (mg/10 g diet)	Start	Weeks of exp. 1	2	3	Wt gain at end of exp.
	Wt of 10 larvae (mg)				
None (control: no Co)	6.3	22.5	138.8	204.6	198.3
Nil	5.5	19.8	43.6	73.3	67.8
Mg, 5 mg	5.7	35.6	110.8	197.8	192.1
", 10 "	5.3	37.3	97.1	200.6	195.3
Fe, 5 "	6.4	31.1	98.3	203.5	197.1
", 10 "	6.1	31.6	180.2	267.5	261.4

* Except where specifically stated otherwise, all diets contained 2.0 mg Co.

experiments, and by themselves had little effect on growth of control larvae.

The data of Tables III-V show (1) that Fe completely overcomes growth inhibition in all cases; (2) that quantitatively considered it is most effective against Ni, since restoration of growth in Ni toxicity is complete at the 5 mg level of Fe itself; in Zn and Co toxicities, 10 mg of Fe is necessary. Further, maximal reversals with Mg are obtained only at 5 mg Mg, higher amounts not being more efficient, causing on the other hand, an additive inhibitory effect in Zn toxicity. Unlike Fe, Mg counteracts Co and Ni toxicities, but not Zn toxicity in *Corcyra* larvae.

Discussion. Evidence available hitherto in regard to trace element interrelationships indicates that ion antagonisms are predominantly prevalent in microorganisms and in plants. The data presented herein are interesting, in that they indicate that interrelationships may also be exhibited by higher forms of life.

TABLE IV. Influence of Fe and Mg on Ni Toxicity in *Corcyra* Larvae.

Supplements to diet* (mg/10 g diet)	Start	Weeks of exp. 1	2	3	Wt gain at end of exp.
	Wt of 10 larvae (mg)				
None (control: no Ni)	8.8	63.3	230.1	388.6	379.8
Nil	9.2	30.1	118.1	201.3	192.1
Mg, 5 mg	9.0	45.6	266.0	404.4	395.4
", 10 "	9.2	60.5	181.1	225.3	216.1
Fe, 5 "	9.1	57.3	259.9	421.4	412.3
", 10 "	8.9	63.3	263.1	475.5	466.6

* Except where specifically stated otherwise, all diets contained 2.0 mg Ni.

In *Corcyra*, it would appear that Co, Ni and Zn can antagonize physiologically important metals. Since complete counteraction of growth inhibition due to these metal toxicities is achieved by Fe or Mg, it will be evident that such antagonistic behavior is reflected in and is intimately associated with the overall growth picture. From this point of view, the difference between Zn toxicity on the one hand, and Co and Ni toxicities on the other, is noteworthy; in Zn toxicity Fe but not Mg can restore growth to normal. Sivarama Sastry and Sarma(13) have shown that in *Corcyra* as in rats, Zn toxicity depresses catalase levels. Moreover, recently Cox and Harris(9) have shown that in rats, the Zn-Fe antagonism manifests itself in altered tissue Fe levels which can be partially brought to normal by additional Fe, though growth itself remains unchanged. The present results emphasize that beneath an apparent formal similarity, metal interrelationships are complex and species specific. It would also appear that in *Corcyra* larvae, the effects of Zn toxicity are attributable to a conditioned Fe deficiency. Further, from the standpoint of comparative biochemistry, this feature constitutes a point of divergence between *Corcyra* larvae and a lower organism such as the mold *Aspergillus niger* wherein Zn toxicity has been recently shown to be equivalent to a Mg deficiency(6).

TABLE V. Influence of Fe and Mg on Zn Toxicity in *Corcyra* Larvae.

Supplements to diet* (mg/10 g diet)	Start	Weeks of exp. 1	2	3	Wt gain at end of exp.
	Wt of 10 larvae (mg)				
None (control: no Zn)	5.7	30.4	104.1	213.2	207.5
Nil	5.5	22.2	49.3	115.9	110.4
Mg, 5 mg	5.3	24.0	66.6	131.4	126.1
", 10 "	6.1	21.2	63.9	72.5	66.4
Fe, 5 "	5.3	28.6	79.0	180.8	175.5
", 10 "	6.1	32.9	120.5	222.4	216.3

* Except where specifically stated otherwise, all diets contained 9.0 mg Zn.

The influence of Mg (Tables III, IV and

V) on metal toxicities is important for another reason also. From the present data it is difficult to say whether the effect of Mg in Co and Ni toxicities is primarily indicative of a Mg deficiency since both Fe and Mg can reverse these toxicities. However, it is possible that the effect of each of these 2 metals, *viz.*, Fe and Mg, is itself dependent on the physiological status of the organism with respect to the other metal. For instance, it has been found recently that in the mold *N. crassa*(5), wherein the level of Mg determines the concentration at which Co, Ni or Zn becomes toxic, reversal of these toxicities by Fe requires a very much higher amount of Fe on a low-Mg medium as compared to one wherein the Mg content is 10-fold higher.

It may be conjectured that excess Fe and Mg influences the intracellular accumulation of toxic metals by tissues. In this context, the demonstration by Abelson and Aldous(3) of such a control of metal ion uptake in certain organisms is of interest.

In any event, the present data indicate that the toxicity of Co, Ni and Zn is associated with their interference with the functioning or utilization of physiological active elements like Fe and Mg. It is likely that such derangements, in turn, bring about far reaching metabolic defects by affecting the rate of synthesis or degradation of vital metabolites. The complete reversal of Zn toxicity in *Corcyra* larvae by nucleic acids without any concomitant effect on accumulation of Zn^{65}(12) is indicative of such a possibility.

Summary. The range of toxicity of Co and Ni to *Corcyra* larvae has been determined. It has been shown that under conditions wherein excess Co, Ni and Zn produce severe inhibition of growth in these larvae, supplementation with Fe uniformly restores growth to normal in all 3 metal toxicities and that Mg is effective only in Co and Ni toxicities. The significance of these results in relation to metal interrelationships in *Corcyra* larvae has been discussed.

1. Hewitt, E. J., *Nature,* 1948, v161, 489.
2. Löhnis, M. P., *Plant and Soil,* 1960, v12, 339.
3. Abelson, P. H., Aldous, E., *J. Bacteriol.,* 1950, v60, 401.
4. Healy, W. B., Cheng, S., McElroy, W. D., *Arch. Biochem. and Biophys.,* 1955, v54, 206.
5. Sivarama Sastry, K., Adiga, P. R., Venkatasubramanyam, V., Sarma, P. S., 1960, Abst. 182, Vth Intern. Congr. Nutr., Washington, D.C., p40.
6. Adiga, P. R., Sivarama Sastry, K., Venkatasubramanyam, V., Sarma, P. S., *Biochem. J.,* 1961, v81, 545.
7. Smith, S. E., Larson, E. J., *J. Biol. Chem.,* 1946, v163, 29.
8. van Reen, R., Pearson, P. B., *Arch. Biochem. and Biophys.,* 1953, v46, 337.
9. Cox, D. H., Harris, D. L., *J. Nutr.,* 1960, v70, 514.
10. Sarma, P. S., *Souvenir Soc. Biol. Chem. (India),* 1955, p49.
11. Adiga, P. R., Sarma, P. S., *J. Sci. and Ind. Res.,* 1961, v20A, 319.
12. Sivarama Sastry, K., Radhakrishnamurthy, R., Sarma, P. S., *Biochem. J.,* 1958, v69, 425.
13. Sivarama Sastry, K., Sarma, P. S., *Nature,* 1958, v182, 532.

Received October 2, 1961. P.S.E.B.M., 1962, v109.

Thyroid State and Glucose Oxidation by Blood.* (27134)

T. W. REDDING AND P. C. JOHNSON
Department of Medicine, Baylor University College of Medicine and The Methodist Hospital, Houston, Texas

The *in vitro* glucose oxidation rate of erythrocytes is changed by prior treatment of the animal with triiodothyronine and thyroxine(1,2). Oxidation is increased during the first 4 days of treatment with high doses of triiodothyronine, but continued treatment causes the oxidation rate to decrease until it reaches 62% of normal by the 21st day when thyrotoxic symptoms first appear(2). *In vitro* addition of physiological doses of triiodothyronine to solutions containing eryth-

* Supported in part by USPHS Grant.