Studies on Zinc Toxicity in the Larvae of the Rice Moth, *Corcyra cephalonica* St.

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Zinc salts fed at relatively high concentrations in the diet have been known to produce deleterious effects. Sutton & Nelson (1938) found that 1% of zinc as carbonate impaired the growth and general health of rats. Smith & Larson (1946) were, however, the first to study extensively this phenomenon in rats. They established that this condition manifested itself in the following two distinct and unrelated symptoms: (*a*) an anaemia, of the microcytic, hypochromic type, and (*b*) an inhibition of growth. The former syndrome could be prevented by supplementing the diet with low levels of copper, and liver extract could partially reverse the adverse influence on growth. van Reen (1953) found that at the 0·5–0·7% level, zinc reduced liver catalase and cytochrome oxidase and that copper could counteract this influence of zinc. These studies indicated that toxic levels of zinc interfere with the utilization of copper and thus indirectly with the formation of iron-containing enzymes. van Reen & Pearson (1953) concluded, however, that the toxicity of zinc was not primarily due to disturbances in these enzyme levels. Such a conclusion is supported by the fact that serious impairement of growth occurs before reduction in any of the enzymes studied.

Thus the underlying mechanisms in zinc toxicosis are not fully known. The situation is complex and although the studies of Smith & Larson (1946) have shown that liver extract is beneficial in zinc toxicosis, the factors responsible have not been characterized, nor is anything known concerning zinc toxicity in other species. In this paper, the results of investigations on zinc toxicity in the lepidopterous insect *Corcyra cephalonica* St. are presented. Although this organism is much lower in the evolutionary scale, it shows striking similarities (Sarma, 1955) to higher organisms like the rat in its nutritional requirements and metabolism.

EXPERIMENTAL

Materials. Zinc was added to the larval diets generally as zinc sulphate ($ZnSO_4,7H_2O$) except in the first set of experiments (Table 1), where zinc chloride was used. Both the salts were products of Merck and Co. Ltd.

All the vitamins used in the growth experiments were purchased from Chas. Pfizer and Co., U.S.A.

Deoxyribonucleic acid and ribonucleic acid were commercial products of Schwarz Laboratories Inc., New York, N.Y., U.S.A.

Liver extract was prepared in the laboratory from sheep liver by autolysis followed by digestion with papain (Mistry, Gajjar & Sreenivasaya, 1945) to yield a final solution containing 100 mg. of total solids/ml. A commercial concentrate (product of Teddington Chemical Factory Ltd., Andheri, Bombay) prepared from the same source was also employed in one set of experiments (Table 3). In this extract 1 ml. was equivalent to 15 g. of proteolysed sheep liver. The alkali-stable fraction of liver extract was prepared by adjusting the extract to pH 11–12 and autoclaving it for 30 min. at 120° (Shenoy & Ramasarma, 1955).

Larval diets and growth studies. Young (10–15 days-old) larvae of the rice moth were used in all experiments. The procedure employed in rearing the larvae was essentially the same as in earlier studies (Sivarama Sastry, 1955).

The basal diet consisted of 10 g. of sieved wheat flour contained in 9 cm. Petri dishes. Supplements, whenever added, were mixed well with wheat flour by using 10–20 ml. of water to achieve uniform distribution. The diets were then allowed to dry at 60° overnight and finely powdered. Thirty larvae were usually reared in each dish.

In all investigations, batches of ten larvae were picked from each dish and weighed every week. The growth data were recorded as the average weights of groups of ten larvae in milligrams. A difference of over 10% in larval weights was always reproducible and hence was considered significant. Growth was studied over 2–3 weeks depending upon the initial size of the larvae, the period being such that in each case the life span of the larval stage from the commencement of the experiment was covered. Where possible, larvae of the same weight were used for each group of experiments. When the initial weights were different, separate control groups were included and all growth data were evaluated on the basis of the appropriate control group.

Experiments with ^{65}Zn. In studies with radioactive zinc, 43 μg. of zinc as $^{65}ZnCl_2$ (supplied by the Radiochemical Centre, Amersham, Bucks.), with a total radioactivity of 9000 counts/min., was added to each gram of diet and the diets were prepared as usual. Larvae were allowed to grow for 3 weeks on the usual diets and then given diets containing ^{65}Zn for 2 days. The larvae were cleaned with a fine brush till completely free of adhering diet and ground up with water. Samples were plated out for measurement of

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radioactivity so that each planchet contained between 30 and 40 mg. of dry larval tissue. ^{65}Zn was determined with an end-window Geiger–Müller tube (2·1 mg. of mica/cm.^{2}) attached to a decade-type autoscaler (Panax Equipment, Mitcham, Surrey). All samples were counted with an accuracy of $\pm 2\%$. No self-absorption corrections were made.

RESULTS

The effects of various levels of dietary zinc on the growth and survival of rice-moth larvae are presented in Table 1.

The various levels of zinc chloride were chosen to determine broadly the range within which zinc is toxic to the organism. It can be seen that at 0·08 and 0·16 % levels of zinc chloride (representing respectively 400 and 800 p.p.m. of zinc) there is no marked effect on growth or survival. The latter level is obviously near the threshold beyond which zinc becomes deleterious, since a very marked inhibition of growth with a concomitantly high mortality was obtained with 0·8 % of zinc chloride. When the level of zinc chloride was doubled to 1·6 % the larvae barely survived for 1 week. The pattern was the same when zinc sulphate was used instead of zinc chloride; in this case the results obtained at a level of 0·1 % were similar to those with 0·08 % of zinc chloride and 1 % was found to be lethal by the end of the third week.

Since Smith & Larson (1946) had shown, in rats, that liver extract could partially reverse the inhibition of growth due to zinc toxicosis it was decided to study whether liver extract was beneficial in the larvae as well. Vitamin B_{12} was also tested at the same time, in view of its importance as one of the anti-anaemic principles of liver extract, and its ability to alleviate the symptoms of metal toxicities, namely, molybdenum toxicity in *Lactobacillus leichmannii* (Daniel & Gray, 1953) and lead anaemia in rabbits (van Klavern & Shrivastava, 1954). The results are summarized in Table 2.

It can be seen that both vitamin B_{12} and liver extract reduced the mortality due to lethal doses of dietary zinc; but liver extract used at a level to provide the same vitamin B_{12} concentration was more effective, suggesting the presence of factors other than this vitamin in liver extract which are involved in this process. It may be seen, however, that in this acute condition, neither liver extract nor vitamin B_{12} restored the growth of the larvae to normal. Subsequently a sublethal, growth-inhibitory level of 0·4 % of zinc sulphate was chosen as the most suitable concentration for further experiments.

The effect of liver extract and of the alkali-stable fraction on the growth of the larvae on a diet with 0·4 % of zinc sulphate was investigated. The results obtained are shown in Table 3.

It is evident that liver extract reverses the inhibition of growth to a very large extent, and that the same result is obtained with the alkali-stable fraction, which accounts quantitatively for the activity of liver extract. In view of this observation other B vitamins were tried for their effective-

Table 1. *Toxicity of zinc chloride to rice-moth larvae*

Expt. no.	Concn. of $ZnCl_2$ in diet (%)	Wt. of 10 larvae (mg.) At transfer	Week 1	Week 2	Week 3	Mortality at the end of expt. (%)
1	0 (control)	8·8	33	141	257	0
2	0·08	8·6	43	122	276	0
3	0·16	8·9	35	113	235	0
4	0·80	8·2	10	13*	14*	78
5	1·60	8·2	2*	—†	—†	100

* Values recorded for surviving larvae. † No survivors.

Table 2. *Influence of liver extract and vitamin B_{12} on growth and survival of rice-moth larvae in zinc toxicosis*

Supplements to basal diet (10 g. of wheat flour)	Wt. of 10 larvae (mg.) At transfer	Week 1	Week 2	Week 3	Survival at the end of expt. (%)
None (control)	5·1	37	120	244	100
Zinc sulphate (0·1 g.)	5·0	6	4*	2*	7
Zinc sulphate (0·1 g.) + vitamin B_{12} (10 μg.)	4·8	10	12	18*	47
Zinc sulphate (0·1 g.) + liver extract†	5·2	12	19	43	70

* Values recorded for surviving larvae.
† Liver extract prepared from sheep liver (see text) was added to provide 10 μg. of vitamin B_{12} (assayed with *Lactobacillus leichmannii* A.T.C.C. 7830 (Radhakrishna murty & Sarma, 1957).

ness in overcoming the inhibition of growth. The results of a typical experiment are presented in Table 4.

Of all the vitamins tested, only vitamin B_{12} and thiamine were effective, even though the restoration of growth was far from complete. It may be mentioned that the levels of the vitamins in this set of experiments were in excess of the normal nutritional requirements of this organism. The growth responses recorded in Table 4 represent the maximum obtained with the various vitamins. In view of the growth-promoting effect of the alkali-stable fraction of liver extract and the comparative ineffectiveness of the B vitamins it seemed worthwhile to study whether the deoxyriboside fraction of liver extract was involved. For this purpose, the influence of deoxyribonucleic acid was examined, and in the same experiment the influence of ribonucleic acid was also studied.

The results (Table 5) indicate that deoxyribonucleic acid (DNA) is very effective at the 0·5% level, maximal reversal occurring at the 1·0% level. It can be seen further that ribonucleic acid is similarly effective. It would thus appear that the synthesis or metabolic activity of the nucleic acids is adversely affected in zinc toxicosis in this organism and that the activity of the liver extracts might be due also to their content of nucleic acids or nucleotides. However, it seemed possible that DNA might make zinc unavailable to the larvae by chelating with the metal (Neuberg & Roberts, 1949). To investigate this possibility larvae were grown on wheat flour (control diet), wheat flour containing 0·4% of zinc sulphate, or wheat flour supplemented with 0·4% of zinc sulphate and 1·0% of DNA, for a period of 3 weeks as in the experiments recorded in Table 5. At the end of this period larvae from each dish were removed and placed for 2 days on diets containing ^{65}Zn alone or ^{65}Zn and DNA, and their ^{65}Zn content was determined as mentioned earlier.

It may be observed from the results in Table 6 that the same amount of zinc is taken up by the larvae whether zinc was supplied alone or in conjunction with DNA, ruling out the possibility that DNA was making zinc unavailable to the organism.

Table 3. *Influence of liver extract and the alkali-stable fraction on inhibition of growth due to zinc toxicosis*

All diets, except no. 1, contained 0·4% of $ZnSO_4,7H_2O$.

Diet no.	Supplements to 10 g. of basal diet	Wt. of 10 larvae (mg.) At transfer	Week 1	Week 2	Wt. gain (mg./10 larvae)
1	None (control, no zinc sulphate)	16	75	287	271
2	None	17	50	158	141
3	2 ml. of liver extract*	17	61	229	212
4	2 ml. of liver extract (alkali-treated)*	16	69	234	218

* Commercial liver extract (see text).

Table 4. *Influence of vitamins of the B complex on zinc toxicosis in rice-moth larvae*

Unless otherwise indicated all diets contained 0·4% of $ZnSO_4,7H_2O$.

Supplements to 10 g. of basal diet	Wt. of 10 larvae (mg.) At transfer	Week 1	Week 2	Week 3
	Experiment *A*			
None (control; no zinc sulphate)	6·7	46	138	237
None	6·6	29	53	127
Vitamin B_{12} (10 μg.)	5·9	34	82	143
Thiamine (1 mg.)	7·8	31	84	157
Pyridoxine (1 mg.)	6·1	29	58	112
Calcium pantothenate (10 mg.)	6·2	29	57	124
Inositol (10 mg.)	6·6	29	56	120
Nicotinic acid (2 mg.)	6·4	30	58	126
Biotin (10 μg.)	6·1	28	55	125
p-Aminobenzoic acid (10 mg.)	6·5	33	55	119
	Experiment *B*			
None (control; no zinc sulphate)	15·0	54	140	253
None	14·0	34	72	111
Folic acid (10 μg.)	14·7	37	68	117
Riboflavin (10 μg.)	13·7	32	62	116
Choline chloride (2 mg.)	16·0	36	63	110

Table 5. *Influence of deoxyribonucleic acid and ribonucleic acid on zinc toxicity in rice-moth larvae*

Unless otherwise indicated, all diets contained 0·4% of $ZnSO_4,7H_2O$.

Supplements to 10 g. of basal diet	Wt. of 10 larvae (mg.) At transfer	Week 1	Week 2	Week 3	Wt. gain (mg./10 larvae)
None (control; no zinc sulphate)	4·0	36	125	198	194
None	4·0	22	60	103	99
0·5% of deoxyribonucleic acid	3·6	21	106	173	169·4
1·0% of deoxyribonucleic acid	3·6	32	132	189	185·4
2·0% of deoxyribonucleic acid	3·5	32	114	184	180·5
1·0% of ribonucleic acid	6·0	42	110	197	191

Table 6. *Influence of deoxyribonucleic acid on uptake of* ^{65}Zn *by tissues of the rice-moth larvae in zinc toxicosis*

Initial larval diet	Uptake of ^{65}Zn by larval tissues (counts/min./100 mg.) With ^{65}Zn alone	With ^{65}Zn and DNA*
Control (no zinc sulphate)	258·8	245·6
0·4% of zinc sulphate	155·2	145·5
0·4% of zinc sulphate and 1·0% of DNA	181·5	179·5

* DNA was added, in each, to provide the same ratio of zinc to DNA as in the growth experiments.

DISCUSSION

The present investigation has shown that in the larvae of the rice moth, *Corcyra cephalonica* St., the inhibition of growth induced by 0·4% of zinc sulphate is partially reversed by either whole-liver extract or its alkali-stable fraction. None of the B vitamins tested was as effective. Thus it would appear that in zinc toxicosis the vitamin status of this organism is mostly unaffected. The behaviour of vitamin B_{12} in reducing the mortality but not the inhibition of growth could be explained on the basis of its possible involvement in nucleic acid synthesis in the larvae, particularly in view of the influence of the nucleic acids on zinc toxicity. It is also possible that the vitamin B_{12} status of the larvae is affected by lethal levels of dietary zinc but not by the lower ones.

The results presented in Table 6 show that DNA, at a level at which it reverses the inhibition of growth completely, does not affect the assimilation of ^{65}Zn to any extent, as the uptake of ^{65}Zn by each of the three groups of the larvae is the same both in the presence and in the absence of DNA. It is likely that in zinc toxicosis in this organism the need for either the nucleic acids as such, or for some of the structural constituents of the DNA molecule, is increased and that this situation brings about the observed inhibition of growth. Further studies would be necessary to determine how the synthesis or metabolism of these compounds is affected in zinc toxicity.

SUMMARY

1. The levels at which dietary zinc becomes toxic to rice-moth larvae have been determined. At lethal levels of zinc, supplementation of the diet with vitamin B_{12} or liver extract checked mortality.

2. None of the B vitamins, except thiamine and vitamin B_{12}, was effective in reversing the inhibition of growth induced by 0·4% of zinc sulphate. Vitamin B_{12} was more effective in prolonging the survival of the larvae than in promoting growth in this condition.

3. Liver extract, as well as its alkali-stable fraction, partially reversed the inhibition of growth due to zinc toxicity.

4. Deoxyribonucleic acid and ribonucleic acid at 0·5–1·0% levels in the diet reversed the inhibition of growth due to zinc toxicity completely. Dietary DNA did not influence the uptake of ^{65}Zn from the diet. The results are discussed.

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