# DEPRESSION OF DNA SYNTHESIS IN THE THYMUS OF ZINC-TREATED ADULT MICE

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$Zn^{2+}$-ions in a high dose affect the uptake of thymidine into thymus DNA. The depression of DNA synthesis in the thymus of zinc-treated mice is dose-dependent and is more pronounced in adult than in young animals.

Zinc is required for the catalytic activity of over one hundred different enzymes, including those involved in the synthesis of nucleic acids[1,2]. Increased requirements for zinc during pregnancy, lactation, growth, development and various chronic disorders[3,4] contribute to marginal zinc status among human population[5]. Deprivation of zinc has been shown to alter beside other processes immunocompetence in both experimental animals and humans[6,7], and animals deprived of zinc markedly reduce their response to T-cell mitogens and thymic-dependent antigens[8]. Such functional changes occurred simultaneously with the atrophy of the thymus[9].

In this report we describe a block in the synthesis of DNA in the thymus of zinc-treated mice. The depression of thymidine uptake into DNA was observed in the thymus of adult animals and not in the spleen or other tissues.

## EXPERIMENTAL

Groups of 6—8 female mice (15 or 25 g) kept under standard conditions were used. Experiments were started at 9 a.m., and the animals were killed by cervical dislocation. Thymidine-[2-$^{14}$C] (1·85 GBq/mmol) delivered by the Institute for Research, Production and Uses of Radioisotopes was injected *i.p.* 2 h before killing at a dose of 37 KBq/0·2 μmol *per animal*. The excised tissues were cooled and immediately homogenized in 2 volumes of distilled water. Procedure for the isolation of radioactive thymine from the total DNA present in homogenates was the same as described earlier[10]. The rate of DNA synthesis is expressed as the specific radioactivity of thymine-[2-$^{14}$C] in dpm/μmol. Zinc, nickel, cobalt and cadmium chlorides were of analytical grade purity.

## RESULTS AND DISCUSSION

Previously we followed[11] the effect of metal ions on the rate of DNA synthesis in regenerating rat livers. In this study we measured the effect of several heavy metals on the synthesis of DNA in various tissues of mice (Table I). Similar to cadmium and 5-aza-2′-deoxycytidine (a specific inhibitor of DNA synthesis in the lymphatic system[12]) the administration of zinc at relatively high dose level results (Table II) in the lowering of thymidine incorporation into DNA in the thymus of metal-treated mice. The synthesis of DNA in other tissues, especially in the spleen, was unchanged.

TABLE I

Rate of DNA synthesis in various tissues of mice treated with heavy metals. Groups of 5 female mice (25 g) received *i.p.* 24 h before killing chlorides of individual metals at the dose of 5 mg per kg. Thymidine-[2-$^{14}$C] (37 KBq/0·2 μmol *per animal*) was injected *i.p.* 2 h before killing

| Tissue | DNA synthesis, dpm/μmol ± S.E. | | | | |
|---|---|---|---|---|---|
| | control | $Ni^{2+}$ | $Zn^{2+}$ | $Mn^{2+}$ | $Co^{2+}$ |
| Thymus | 5 740 ± 260 | 4 800 ± 285 | 2 440 ± 310 | 3 070 ± 170 | 1 980 ± 100 |
| Spleen | 15 700 ± 1 320 | 19 150 ± 680 | 16 750 ± 1 000 | 16 680 ± 1 310 | 19 600 ± 625 |
| Lung | 2 360 ± 340 | 1 710 ± 275 | 2 110 ± 125 | 1 580 ± 265 | 2 905 ± 130 |
| Kidney | 3 180 ± 120 | 1 950 ± 310 | 3 160 ± 180 | 3 850 ± 75 | 2 970 ± 145 |

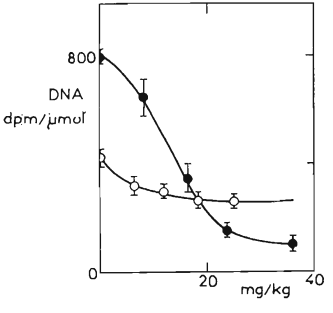


FIG. 1

DNA Synthesis in the thymus of young (○) and adult (●) zinc-treated mixe. Two sets of groups of 6 female mice (15 and 25 g, resp.) were injected *i.p.* 24 h before killing with $ZnCl_2$ (mg/kg) and 22 h later with thymidine-[2-$^{14}$C] (37 KBq/0·2 μmol per 15 g mouse and 56 KBq/0·3 μmol per 25 g mouse, resp.)

TABLE II

Lower thymidine incorporation into DNA in the thymus of mice treated with zinc, cadmium and 5-aza-2′-deoxycytidine. Groups of 6 female mice (25 g) were injected 24 h before killing with the compound; thymidine-[2-$^{14}$C] (37 KBq/0·2 μmol *per animal*) was given 22 h later

| Administered | Dose mg/kg | DNA synthesis, dpm/μmol ± S.E., % | |
|---|---|---|---|
| | | thymus | spleen |
| Control | 0 | 4 100 ± 184 (100) | 12 500 ± 1 105 (100) |
| $CdCl_2$ | 4 | 855 ± 66 (20·8) | 9 170 ± 368 (73·5) |
| $ZnCl_2$ | 16 | 625 ± 103 (15·2) | 17 440 ± 1 785 (139) |
| 5-AzCdR | 3 | 514 ± 20 (12·5) | 3 980 ± 215 (31·8) |
| $CdCl_2$ + 5-AzCdR | 4 + 3 | 248 ± 32 (7·6) | 2 120 ± 110 (17·0) |
| $ZnCl_2$ + 5-AzCdR | 16 + 3 | 464 ± 58 (11·3) | 4 930 ± 388 (39·5) |
| $CdCl_2$ + $ZnCl_2$ | 4 + 16 | 469 ± 47 (11·4) | 9 840 ± 1 037 (78·7) |

However, the combination of cadmium with the analogue further enhanced the depression of the DNA synthesis in both tissues under study. The concomitant administration of cadmium and zinc led essentially to similar results as with either substance given alone (Table II).

There is a marked difference in the response of young and adult mice to the inhibitory action of zinc (Fig. 1). In young animals the administration of zinc did not affect DNA synthesis in the thymus and the spleen considerably; however, in adult animals DNA synthesis in the thymus was depressed by 90%. In both young and adult mice there was no change in the activity of thymidine and thymidylate kinases measured in cell-free extracts of the thymus and spleen, and there was no alteration on the uptake of orotic acid into liver RNA.

The observation of marginal status of zinc ions in humans[5] coupled with findings of altered immunocompetence and impaired response of different pathogenic factors[7] led us to follow the action of zinc administered in relatively high dose levels. The depression of DNA synthesis in zinc-treated mice seems to be rather specific for the thymus tissue of the adult animals. It is obvious from our data that the inhibitory role of zinc in the thymus is maximal at the time of physiologic depletion of thymus cellularity. Further study of the role of zinc in the thymus function is in progress.

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