The Effect of Zinc Deficiency on the Timing of Deoxyribonucleic Acid Synthesis in Regenerating Rat Liver

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SUMMARY

The effect of a short-term (3-day) dietary zinc deficiency on DNA synthesis in regenerating rat liver was investigated, with particular attention being paid to the timing of the S-phase of synthesis. The findings indicated a significantly reduced ($P<0,01$) incorporation of ^{3}H-thymidine into the DNA of animals receiving the zinc-deficient ration (0,3 μg/g) when compared with control animals which received 60 μg/g in their diet. Of special interest was the finding that a shift occurred in the timing of the peak of maximum incorporation, from 17½ hours postoperatively in the control animals to 25 hours postoperatively in the deficient animals. Thus, when comparisons were made between the incorporation data at the respective peaks of maximum DNA synthesis, the effect of zinc deficiency was considerably reduced ($P<0,05$), but not eliminated when compared with the data obtained at the same time postoperatively in both groups.

The data highlight the need for studies concerning the effect of zinc deficiency on the incorporation of ^{3}H-thymidine to be performed at the peak of maximum DNA synthesis for the respective treatments, and not, as is done at present, at the same time for all groups.

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The importance of a regular dietary intake of zinc in animal nutrition has recently become increasingly evident.[1-5] Prolonged depletion leads to the development of a characteristic deficiency syndrome in most animals,[6,7] but even a brief restriction results in impaired cell division and growth in certain tissues.[8,9] Many deficiency symptoms have been attributed to the role of zinc in a number of metallo-enzyme systems,[10,11] but the rapid effect of the depletion on growing tissue suggests a primary locus of action for zinc that is affected before the appearance of the overt symptoms. Little is known at present concerning this primary lesion,[12,13] although evidence has recently been presented suggesting impaired DNA synthesis in zinc-deficient animals.[13-17] No attention has, however, been paid to the effect of zinc deficiency on the timing of DNA synthesis during cell division.

The present study was designed to establish the effect of a short-term (3-day) zinc deficiency on DNA synthesis in regenerating rat liver, and to establish the timing of the S-phase (DNA synthesis) of cell division in both control and zinc-deficient animals.

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MATERIALS AND METHODS

Animals and Diets

Female rats (Wistar strain, weighing 110 - 120 g) were housed in groups of 3 in stainless steel cages and fed a ration consisting of (g/100 g): sucrose 51; soya bean meal (44% protein) 38,5; maize oil 6,1; salt mix[5] 4,0; cod liver oil 0,7; DL-methionine 0,5; choline chloride 0,2. The soya bean meal was extracted with ethylenediamine tetra-acetic acid to reduce its zinc content,[18] and the entire ration contained less than 0,35 μg/g of zinc. Control animals received the same ration supplemented with 60 μg/g of zinc as zinc sulphate. A mixture of crystalline vitamins was supplied separately in sucrose three times a week.[5]

Surgical Procedure

Partial hepatectomy (70%) was performed according to the method of Higgins and Anderson.[19]

Plasma Zinc

Blood samples were collected directly from the heart at the time the animals were killed, and plasma zinc levels were determined by atomic absorption spectroscopy using a Varian Techtron 1000 atomic absorption spectrophotometer.[20]

Incorporation of ^{3}H-thymidine

DNA synthesis was studied by measuring the incorporation of ^{3}H-thymidine (methyl-T) into DNA isolated from regenerating livers of control and zinc-depleted rats at intervals of 2½ hours between 10 hours and 30 hours

postoperatively. The animals received a single intraperitoneal injection of ^{3}H-thymidine (25 mCli) 60 min before the livers were removed, and the specific activity of the DNA was determined by the method of Fujioka and Lieberman.[14]

Because of the difficulty in interpreting incorporation data, certain preliminary studies were made on groups of stock colony female rats (110 - 120 g) to establish that the data would reflect only active DNA synthesis during the incorporation period. Initially, a comparison was made between the amount of incorporation of label occurring in 60 min in partially-hepatectomised animals 20 hours postoperatively, with that in animals 20 hours after receiving only a sham operation. In addition, DNA was isolated 5 min after injection of the ^{3}H-thymidine in order to establish the degree of non-specific binding of the isotope to the weakly-labelled, isolated DNA.

RESULTS AND DISCUSSION

The results of the preliminary investigation (Table I) indicate that incorporation of ^{3}H-thymidine was increased approximately eight-fold 20 hours postoperatively in partially-hepatectomised stock colony rats when compared with animals subjected to only a sham operation. The specific activity of DNA isolated from regenerating liver 5 min after injecting the isotope was reduced to only 5% of that found in animals allowed a 60-min period of incorporation, which indicates that there was very little contamination of the isolated DNA by unincorporated ^{3}H-thymidine.

TABLE I. INCORPORATION OF ^{3}H-THYMIDINE INTO LIVER DNA OF STOCK COLONY RATS

Treatment	No. of rats	Incorporation of ^{3}H-thymidine (cpm/mg DNA)
Partial hepatectomy	4	45 879 ± 4 429
Sham operation	4	5 864 ± 336
5-min incorporation period	4	2 236 ± 501

Data concerning the relative incorporation of ^{3}H-thymidine into the regenerating livers of zinc-deficient and control rats (Table II), indicate that the incorporation was significantly reduced ($P<0,01$) in the deficient animals from 15 hours postoperatively.

TABLE II. INCORPORATION OF ^{3}H-THYMIDINE INTO THE LIVERS OF ZINC-DEFICIENT AND CONTROL RATS*

Status of rat	Time postoperatively (h)	Plasma zinc (μg/ml)†	Incorporation of ^{3}H-thymidine (cpm/mg DNA)
Control	10	1,04 (1,07 — 0,92)	10 728 ± 481
Zinc-deficient	10	0,49 (0,58 — 0,37)	10 309 ± 840
Difference of means			419 ± 613
LSD (0,25)‡			430
Control	15	1,03 (0,89 — 1,11)	33 174 ± 2 302
Zinc-deficient	15	0,51 (0,47 — 0,59)	22 000 ± 1 382
Difference of means			11 174 ± 1 692
LSD (0,01)			4 738
Control	17½	1,01 (0,89 — 1,08)	46 339 ± 3 841
Zinc-deficient	17½	0,59 (0,41 — 0,72)	31 016 ± 3 975
Difference of means			15 023 ± 3 478
LSD (0,01)			9 405
Control	20	1,00 (0,91 — 1,07)	45 879 ± 4 429
Zinc-deficient	20	0,54 (0,43 — 0,61)	29 917 ± 2 696
Difference of means			15 962 ± 5 182
LSD (0,01)			15 148
Control	22½	1,04 (0,92 — 1,10)	44 746 ± 2 504
Zinc-deficient	22½	0,56 (0,43 — 0,71)	29 027 ± 3 775
Difference of means			15 719 ± 271
LSD (0,01)			7 674
Control	25	1,01 (0,89 — 1,09)	41 373 ± 1 682
Zinc-deficient	25	0,58 (0,51 — 0,70)	35 429 ± 1 414
Difference of means			5 944 ± 1 269
LSD (0,01)			3 942
Control	30	1,05 (0,89 — 1,14)	36 408 ± 1 063
Zinc-deficient	30	0,57 (0,43 — 0,61)	31 386 ± 1 783
Difference of means			5 022 ± 1 194
LSD (0,01)			3 243

* Data from 6 animals in each group.
† Mean values, range given in parentheses.
‡ Student's *t*-test.

The findings relating to the timing of the peak of maximum thymidine incorporation are of particular interest (Table III). In the control group, maximum incorporation occurred between 17½ hours and 20 hours

TABLE III. INCORPORATION OF ^{3}H-THYMIDINE INTO THE LIVERS OF ZINC-DEFICIENT AND CONTROL RATS*

Status of rat	Time postoperatively (h)	Incorporation of ^{3}H-thymidine (cpm/mg DNA)	Difference of means
Control	**17½**	**46 339 ± 3 811**	**4 966 ± 2 538**
Control	**25**	**41 373 ± 1 682**	
			LSD (0,05) 4 428†
Zinc-deficient	**17½**	**31 016 ± 3 975**	**4 413 ± 2 571**
^{3}H-thymidine	**25**	**35 429 ± 1 414**	
			LSD (0,05) 4 528
Control	**17½**	**46 339 ± 3 841**	**10 910 ± 2 478**
Zinc-deficient	**25**	**35 429 ± 1 414**	
			LSD (0,01) 9 150

* Data from 6 animals in each group.
† Student's *t*-test.

postoperatively and declined significantly ($P<0,05$) after 25 hours. With the deficient animals, however, incorporation of label rose to be higher at 25 hours than at any other time ($P = 0,05$), which indicates a marked delay (7½ hours) in the time of maximum DNA synthesis (S-phase) in this group. In the present experiment, a significant reduction in DNA synthesis ($P<0,01$) was observed in the zinc-deficient animals at all times between 15 hours and 30 hours postoperatively, even when comparisons were made between the control values at 17½ hours and the deficient data at 25 hours. It must, however, be stressed that the magnitude of the reduction decreased significantly ($P<0,05$) when the latter allowances were made for delayed DNA synthesis. The present finding drew attention to the possibility that in other experiments where the reduction in DNA synthesis after zinc depletion is less marked, data obtained at the respective times of maximum thymidine incorporation for each group may differ widely from those obtained when both groups are studied at the same time.

Clearly, future investigations concerning zinc status and DNA synthesis must take account of the effect of zinc depletion on the timing of the S-phase during mitosis.

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