RIBONUCLEASE ACTIVITY AND NUCLEIC ACID AND PROTEIN METABOLISM IN THE TESTES OF ZINC-DEFICIENT RATS

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[*Manuscript received April* 8, 1969]

Summary

The growth, feed consumption, feed efficiency, testicular weight, and composition of the testes of young rats fed a zinc-deficient (1·4 p.p.m.) diet over a 10-week period were studied and compared with those of two groups of control rats fed this diet supplemented with zinc to provide 90 p.p.m. zinc. The feed intake of one of the control groups was restricted to that of the zinc-deficient group, so that the effects of inanition and of zinc deficiency *per se* could be separated.

The liveweight gains, efficiency of feed conversion, weight of testes, and the ratio of testes weight to liveweight were significantly lower in the zinc-deficient rats than in either the pair-fed or the unrestricted-fed control rats.

The testes of the zinc-deficient rats contained significantly lower concentrations of zinc, RNA, DNA, and protein and significantly higher non-protein-nitrogen concentrations and ribonuclease activity than the testes of either of the control groups.

It was concluded that protein and nucleic acid metabolism is defective in the testes of zinc-deficient rats and that a primary defect is an increase in ribonuclease activity resulting in increased protein catabolism.

I. INTRODUCTION

In plants and microorganisms zinc has been shown to be involved in nucleic acid and protein metabolism (Winder and Denneny 1959; Schneider and Price 1962; Wacker 1962; Winder and O'Hara 1962; Wegner and Romano 1963). The findings of these workers suggest that the earliest biochemical lesion in zinc deficiency is a failure in RNA formation, followed by protein and DNA, with a concomitant increase in non-protein nitrogen and acid-soluble amino acids (Schneider and Price 1962; Wacker 1962). The precise locus of action of zinc in these processes is unknown. Vallee (1959) reported that zinc occurs as a normal constituent of RNA from *Euglena gracilis* and other organisms but the significance of this finding in relation to zinc deficiency is not clear. Yeast ribonuclease can be completely inhibited by zinc at a concentration of 10^{-4}M (Ohtaka, Uchida, and Sakai 1963), which suggests that zinc at physiological concentrations may act through protecting RNA from excessive degradation by nucleases. Such a possibility finds support from the existence of higher ribonuclease activity in zinc-deficient apple and citrus leaves than in zinc-sufficient control leaves (Kessler and Monselise 1959; Kessler 1961).

The results of studies of nucleic acid and protein metabolism in zinc-deficient animals are discordant. Williams *et al.* (1965) reported a reduction of at least 40% in the incorporation of ^{32}P into the nucleotides of RNA and DNA in the livers of zinc-deficient rats, indicating an impairment of nucleic acid metabolism in this organ.

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The isotope studies of Mills *et al.* (1967) and of Theuer and Hoekstra (1966) also provide evidence of defective protein synthesis in zinc-deficient rats. On the other hand, Turk (1966) found the liver concentrations of RNA, DNA, total nitrogen, and acid-soluble nitrogen to remain within normal limits in chicks showing signs of zinc deficiency. Furthermore, Macapinlac *et al.* (1968) were unable to detect a gross impairment of DNA synthesis in the testes of rats in early zinc deficiency. The rate of incorporation of [^{14}C]leucine and [^{14}C]adenine into protein and RNA, respectively, was reported to be unaltered, although the total protein and RNA contents of the testes were reduced in the zinc-deficient rats. The authors suggested that their data were consistent with an increase in protein and RNA catabolism in zinc-deficient testes rather than with a decrease in synthesis.

Preliminary observations made by us indicated that the testes of severely zinc-deficient rats contained appreciably lower levels of RNA, DNA, and protein and higher ribonuclease activities than those of control rats. In the present study the observations were investigated further with larger numbers of animals, including a feed-restricted group, so that the effects of inanition and of zinc deficiency *per se* could be differentiated.

II. Experimental Methods

The mean liveweight of the 36 Wistar strain male rats was 54·7 g at 35 days of age when the experiment started. Each rat was individually held in a cage made of stainless steel and polythene, equipped with a polythene watering bottle and a Pyrex glass feed-holder.

Twelve rats were assigned to each of three treatment groups on a liveweight basis. Rats in group 1 were fed the following low-zinc diet (containing 1·4 p.p.m. zinc):

Spray-dried egg white	25·0%	Glucose	53·3%
Corn oil	10·0%	A.R. grade mineral mix*	3·7%
Cellulose	3·0%	Vitamin–glucose mix*	5·0%

* After Forbes and Yohe (1960). The diet was also supplemented to contain the following per 100 g: 1000 i.u. vitamin A, 150 i.u. vitamin D, and 50 mg α-tocopherol. An additional biotin supplement (1 mg/kg) was added routinely in view of the use of egg white as the protein source.

The zinc content of the diet fed to rats in group 2 was increased to 90 p.p.m. by the addition of zinc sulphate but feed was restricted on a daily basis to individuals in this group corresponding to a pair member in group 1. Group 3 rats were fed the diet containing 90·0 p.p.m. zinc in unrestricted amounts. Deionized water was freely available for drinking. The rats were weighed weekly and were inspected daily for external signs of zinc deficiency.

Typical zinc-deficiency lesions appeared in the rats fed the basal diet by the end of the second week and by the tenth week had become so severe that one rat died in a weak emaciated condition. The corresponding rat in the feed-restricted control group was then eliminated together with one other rat from group 3. In this way even numbers were maintained in each group.

During the eleventh week all remaining rats were killed by concussion and within 1–2 min both testicles were removed and weighed separately, minus the epididymis.

A variation of the procedure described by Dickman, Aroskar, and Kropf (1956) was used for measuring the relative ribonuclease activity in the testes. Homogenates were immediately prepared in a Sorval homogenizer to contain 100 mg of testes per millilitre with 0·25M acetate–0·001% gelatin buffer (pH 5·0). A 1·0-ml aliquot of the homogenate was added to each assay tube and the reaction initiated by adding 1·0 ml of 0·3% yeast RNA (Sigma Chemical Co.) Inactive enzyme controls were also prepared according to Dickman, Aroskar, and Kropf (*loc. cit.*). All tubes were incubated for 30 min at 37°C when the reaction was stopped by adding 3·0 ml of

glacial acetic acid–butanol (1:2 v/v). The mixture was stirred, allowed to stand for 15 min, centrifuged, and 1·0 ml of the nucleotide-containing supernatant appropriately diluted with water to measure the absorbancy at 260 mμ in a Gilford spectrophotometer. The difference in absorbancy between the active and inactive control tubes was used to indicate nucleotide production from the RNA substrate and hence ribonuclease activity.

RNA and DNA were partitioned in weighed portions of the testes using the method of Schmidt and Thannhauser (1945) as modified by Hutchinson, Downie, and Munro (1962). The RNA was determined by ultraviolet absorption at 260 mμ and DNA was estimated by the diphenylamine procedure of Burton (1956) using the two optical density readings of 595 and 700 mμ suggested by Giles and Myers (1965).

The remainder of the testes was homogenized in deionized water and used for (1) zinc determinations by wet ashing and atomic absorption spectroscopy (Allen 1961); (2) total nitrogen determinations by the microKjeldahl method of McKenzie and Wallace (1954); and (3) protein nitrogen, determined similarly on trichloroacetic acid precipitates of the homogenates.

III. Results

Rats in group 1 after 10 weeks of feeding the zinc-deficient diet had lost hair, had hunched backs, and had lesions of the feet and eyes. The liveweight growth curves for this group and the two control groups are shown in Figure 1. These curves

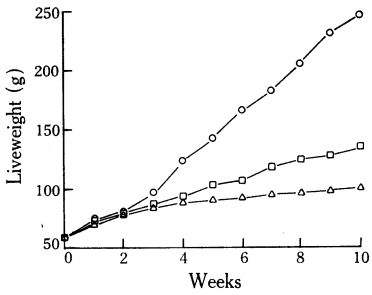

Fig. 1.—Liveweight growth curves of rats fed the zinc-deficient diet (△). □ Pair-fed controls. ○ Unrestricted control diet.

indicate that the rate of growth of the zinc-deficient rats was severely depressed compared with either of the control groups. This differential in the growth rate was evident after the second week and increased in magnitude over the remaining 8 weeks until the rats were killed. Data relating to the feed intake and the growth performance of the rats in the three groups are presented in Table 1.

Rats fed the zinc-deficient diet consumed a significantly smaller amount of food than the *ad libitum*-fed control. Both of the zinc-supplemented groups had a significantly higher efficiency of feed utilization (grams of feed required/grams of liveweight gain) than that of the zinc-deficient group.

Inanition also influenced the testicular weights of the rats in group 2. They weighed significantly less than those from the *ad libitum*-fed control group but were significantly heavier than those from the zinc-deficient rats in group 1. The relative weight of the testes (i.e. as a percentage of body weight) of the zinc-deficient rats was also significantly less than that of the restricted-fed control group.

In addition to the hypogonadism that resulted from feeding the zinc-deficient diet, important differences in the composition of the testes were observed. These are presented in Table 2.

The zinc content of the testes from the deficient rats was significantly lower than that of either of the control groups, a finding consistent with that of other workers (Millar *et al.* 1958; Prasad *et al.* 1967; Barney, Orgebin-Crist, and Macapinlac 1968;

TABLE 1

FEED INTAKE AND GROWTH PERFORMANCE OF ZINC-DEFICIENT RATS (GROUP 1), PAIR-FED CONTROL RATS (GROUP 2), AND *AD LIBITUM*-FED ZINC-SUFFICIENT RATS (GROUP 3)

Group No.	Liveweight when Killed (g)	Liveweight Gain (g)	Dry Matter Intake (g)	Efficiency of Feed Conversion	Weight of Single Testicle (g)	Ratio of Total Testicular Weight to Liveweight (%)
1	102	44·5	499	11·6	0·47	0·92
	±3·6	±3·4	±22·2	±0·74	±0·03	±0·08
2	135	77·6	499	6·6	1·07	1·58
	±4·8	±4·7	±22·2	±0·39	±0·05	±0·10
3	247	194	798	4·1	1·29	1·06
	±5·5	±4·2	±20·0	±0·07	±0·04	±0·03
Least significant differences:						
$P<0·05$	13·3	11·9	62·9	1·4	0·11	0·22
$P<0·01$	17·9	16·0	86·2	1·9	0·15	0·29

TABLE 2

ZINC, RNA, DNA, AND NITROGEN CONTENT AND ALSO RIBONUCLEASE ACTIVITY OF TESTES FROM ZINC-DEFICIENT RATS (GROUP 1), PAIR-FED CONTROL RATS (GROUP 2), AND *AD LIBITUM*-FED ZINC-SUFFICIENT RATS (GROUP 3)

Group	Amount in Testes of:						Ribonuclease Activity†
	Zn (μg/g)	RNA (mg/g)	DNA (mg/g)	Total Nitrogen (mg/g)	Protein Nitrogen (mg/g)	Non-protein Nitrogen* (mg/g)	
1	21·0	3·2	3·0	14·0	8·3	5·7	0·21
	±0·83	±0·16	±0·15	±0·40	±0·32	±0·13	±0·009
2	32·5	5·0	4·5	14·5	10·8	3·8	0·12
	±1·08	±0·14	±0·24	±0·36	±0·28	±0·10	±0·005
3	36·5	5·3	4·7	15·0	11·6	3·4	0·13
	±1·68	±0·19	±0·13	±0·41	±0·38	±0·21	±0·003
Least significant differences:							
$P<0·05$	3·47	0·47	0·51	1·11	0·93	0·43	0·02
$P<0·01$	4·67	0·64	0·68	1·49	1·25	0·59	0·03

* Calculated by difference.

† Absorbancy at 260 mμ due to nucleotide production from RNA by 100 mg of testes.

Macapinlac *et al.* 1968). This was associated with significantly lower concentrations of RNA, DNA, and protein and significantly higher non-protein-nitrogen concentrations and ribonuclease activity in the zinc-deficient testes compared with both control

groups. The zinc concentration in the testes of the two control groups did not differ significantly, nor did the concentrations of RNA, DNA, or protein, despite the differences in the testes weights of these groups. It seems that the cellular mass was reduced in the testes by inanition but nucleic acid and protein metabolism proceeded normally.

When inanition was accompanied by zinc deficiency (group 1) there was a significant reduction in testicular weight compared with that due to inanition alone (group 2). The significantly lower testicular weights in the zinc-deficient group emphasize the importance of zinc for gonadal growth. This is shown further by the lower concentrations of RNA, DNA, and protein found in the zinc-deficient testes compared with the concentrations in control groups.

Although the concentration of protein nitrogen in the testes of the zinc-deficient rats was significantly lower than in either of the control groups, there was no significant difference in the concentration of total nitrogen in the testes between any of the groups. Hence the testes of the zinc-deficient rats contained a significantly higher concentration of non-protein nitrogen. The nature of the nitrogen-containing compounds in this fraction was not determined.

IV. Discussion

Our data indicate clearly that nucleic acid and protein metabolism is defective in the testes of zinc-deficient rats and that this defect is not a manifestation of the inanition of zinc deficiency. These findings support and extend the conclusions of Williams *et al.* (1965) for the livers of zinc-deficient rats and those of Theuer and Hoekstra (1966) and Mills *et al.* (1967) but they are not in accord with those of Turk (1966). This worker observed no changes in the contents of DNA, RNA, total nitrogen, or acid-soluble nitrogen in the livers of chicks after 3 weeks subsistence upon a zinc-deficient diet. In this case, as pointed out by the authors, the time may have been too short for changes in these constituents to appear. The only other experiment known to us in which nucleic acid and protein metabolism in the testes in zinc deficiency was studied is that of Macapinlac *et al.* (1968), who found that the rates of incorporation of [^{14}C]adenine into RNA and of [^{14}C]leucine into protein were unaltered. However, the total protein and RNA contents of the testes were reduced. It was suggested that an increase in the catabolism of these constituents occurred as a consequence of the zinc deficiency. Such a hypothesis finds support from the present study, in which significantly decreased concentrations of zinc, RNA, DNA, and protein nitrogen in the testes of the zinc-deficient animals were associated with significant increases in non-protein-nitrogen concentrations and in ribonuclease activity. It seems that one of the primary functions of zinc is to control ribonuclease activity at the cellular level. At the low testicular zinc concentrations typical of the zinc-deficient rat ribonuclease activity is enhanced, with a consequent increase in protein catabolism in this tissue.

V. Acknowledgments

The authors wish to thank Mrs. Sandra Klyne and Mr. B. Lange for technical assistance and the Australian Research Grants Committee for financial support.

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