Comparisons Between Nucleic Acids, Protein, Zinc, and Manganese in Rat Liver: A Relation Between Zinc and Ribonucleic Acid

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Extract

The relations of total zinc, manganese, nucleic acids, and protein in the livers of control, hypophysectomized, and calorie-restricted rats were evaluated by regression analysis and analysis of covariance (tables I through V).

The most striking findings were the demonstration of a continuous relation between RNA and zinc in the livers of the control rats and of experimental groups (fig. 2), and an absolute increase in the content of manganese in the livers of the calorie-restricted animals (fig. 3).

It is suggested that zinc is important for the maintenance of ribonucleic acid synthesis and subsequent protein synthesis in mammalian tissues.

Speculation

In biological tissues, ribonucleic acid is a necessary precursor for protein synthesis; possibly zinc, acting as a cofactor, is of equal importance.

Introduction

In an earlier paper [3] it was reported that during a variety of circumstances affecting growth of rats (endocrine imbalance, calorie restriction) the ratios in liver of both zinc and ribonucleic acid to deoxyribonucleic acid appeared to parallel each other closely. This suggested that the two components were related within the cell. The present study was designed to examine the relation of zinc and manganese to nucleic acids and protein within the rat liver by more definitive statistical methods in order to strengthen or discard the previous finding.

Statistical analysis, namely regression analysis and analysis of covariance, was utilized to inspect the relation of total zinc (Zn) and also total manganese (Mn) in rat liver with the liver content of deoxyribonucleic

acid (DNA), ribonucleic acid (RNA), and protein. These interrelations are considered for control rats, rats receiving adequate protein but inadequate calories, and for rats that had been hypophysectomized.

Regression equations presented in this communication contain a common factor, liver weight, through which both the dependent and independent variables are calculated. This, of course, leads to somewhat spurious correlations. The emphasis of the statistical analysis, however, is not on the fitted equations but on the comparisons of the sets of data by analysis of covariance. This permits comparisons of animals of varying ages and sizes in a way that is biologically meaningful.

Analysis of covariance affords a sensitive statistical method by which two regression lines may be compared to determine whether they are from two different pop-

ulations or are composed of two samples from the same population. If they are from two different populations, further analysis allows the investigator to decide sequentially if the lines are of different slopes or if they are parallel.

The final analysis indicates a continuous relation between RNA and Zn content under the conditions studied and the biological implications that follow from this relation [29].

Methods and Materials

Control and 'calorie-restricted' animals correspond to groups 1A-1E and group II, respectively, as previously reported [10]; hypophysectomized rats were described as group II in another communication [2].

Briefly, male rats of the Sprague-Dawley strain were obtained at 21 days of age [30]; at 23 days of age, they were placed in individual metabolic cages with tap water freely available.

The control group of this study were fed a laboratory ration [31] (23% protein) *ad libitum* and were killed at 26, 28, 35, 38, and 49 days of age, respectively. The calorie-restricted group received the same ration [31] but restricted in quantity to the intake of hypophysectomized rats that had had their pituitaries removed at 21 days of age; both abnormal groups were killed at 38 and 49 days of age.

The methods used for the determination of nucleic acids, protein, and trace metals are documented elsewhere [5, 10]. The total amounts of DNA, RNA, protein, Zn, and Mn were calculated as the product of the individual liver weight and the concentration of the corresponding individual determinants.

Regression and covariance analysis are described in many texts concerned with statistics. In addition, a summary has been presented elsewhere [15]. We consider an alpha level (*P* value) equal to 0.05 as being of borderline significance and not significant statistically. Levels of *P* < 0.025 were considered significant.

Results

The relations between total amounts of DNA, RNA, protein, Zn, and Mn in the liver of normal rats were determined by regression analysis. It was found that these quantities essentially were linearly related to each other. The correlation coefficients were between 0.90 and 0.97. The linear functions for normal rats are presented in table I. These relations were of sufficient accuracy to allow comparisons with the abnormal groups of rats.

Linear analysis of covariance was used for statistical

Table I. Linear equations, normal rats

Equation	N ¹	Variance ²
RNA ³ , DNA ⁴ , and protein ⁵		
RNA = 10.3834 + 3.0646 (DNA)	32	118.18
RNA = 13.4214 + 0.0529 (protein)	33	45.76
Protein = -110.892 + 59.24034 (DNA)	35	25,353
Zn ⁶		
Zn = -7.03624 + 3.17133 (RNA)	33	971.80
Zn = 14.107 + 0.1851 (protein)	45	634.76
Zn = -7.52743 + 11.04712 (DNA)	44	1,403.33
Mn ⁷		
Mn = 0.72399 + 0.19505 (RNA)	33	3.204
Mn = 1.1973 + 0.012 (protein)	45	2.227
Mn = -0.67934 + 0.74339 (DNA)	44	3.076

¹ N = number of rats in sample.

² Variance = variance about the fitted line.

³ RNA = total liver RNA, mg.

⁴ DNA = total liver DNA, mg.

⁵ Protein = total liver protein, mg.

⁶ Zn = total liver Zn, μ g.

⁷ Mn = total liver Mn, μ g.

Fig. 1. Interrelation of nucleic acids and protein. Solid line represents the control. Dashed line represents the standard deviation. The equation to each normal relation is reported in the text. $\circ$ $\bullet$: hypophysectomized rats. $\square$ $\blacksquare$: calorie-restricted rats. Solid symbols represent significant difference with controls by covariance analysis; open symbols represent nonsignificance. The length of the lines represent the range of data for each group.

comparison of these groups. These analyses are graphically illustrated in figures 1, 2, and 3.

RNA, DNA, and Protein (table II)

Figure 1 shows that calorie-restricted rats consistently had higher total RNA and protein for a given DNA value (*P* < 0.001). Total RNA was slightly high for a given protein value although this difference was not significant statistically.

In the liver of the hypophysectomized rats, the RNA content was reduced significantly from normal values when comparisons were made relative to protein or DNA ($P < 0.001$). The relation between protein and DNA, however, was similar to that of normal rats.

Zinc (table III)

In calorie-restricted rats, the relation between the amounts of Zn and RNA or of Zn and protein were not significantly different from normal animals (fig. 2). In these rats, Zn in the liver was high relative to DNA ($P < 0.001$).

Hypophysectomized rats appeared to have normal

values for Zn relative to RNA and DNA. There was a significant difference ($P < 0.01$), however, in the amount of Zn relative to protein. The points for hypophysectomized rats formed a line that intersected the normal line. Due to the small range of protein values for these rats it is impossible to say whether they really departed from normal.

Thus, the relation between Zn and RNA did not depart from normal in either the calorie-restricted or the hypophysectomized rats. As a result, a linear equation combining all three groups was calculated:

$$\text{Zn} = 18.4149 + 2.7831 (\text{RNA})$$

where $N = 67$ and variance = 761.89.

Table II. Covariance analysis

Source ¹	Control rats versus					
	Hypophysectomized rats			Calorie-restricted rats		
	df ²	Mean square	F	df	Mean square	F
RNA:DNA						
Reduction—fitting two lines	2	870.19	10.2991***	2	1,108.13	11.2540***
Reduction—fitting two slopes	1	2.89	0.0341	1	635.22	6.4512*
Reduction—fitting two means	1	1,737.50	20.5640***			
Pooled error	45	84.49		45	98.47	
SS about two lines						
RNA:protein						
Reduction—fitting two lines	2	886.47	26.0178***	2	152.15	3.7576
Reduction—fitting two slopes	1	27.05	0.7941			
Reduction—fitting two means	1	1,745.88	51.2415***			
Pooled error	46	34.07		46	40.49	
SS about two lines						
Protein:DNA						
Reduction—fitting two lines	2	5,394.61	0.2714	2	225,865.81	10.1624***
Reduction—fitting two slopes				1	43,723.63	1.9673
Reduction—fitting two means				1	408,008.00	18.3576***
Pooled error	58	19,879.03		58	22,225.55	
SS about two lines						

¹ See table I, footnotes 3 through 7.

² df = degrees of freedom.

* $P < 0.025$.

** $P < 0.01$.

*** $P < 0.001$.

Fig. 2. Relation of Zn with DNA, RNA, and protein. Solid line represents the control. Dashed line represents the standard deviation. The equation to each normal relation is reported in the text. ○ ●: hypophysectomized rats. □ ■: calorie-restricted rats. Solid symbols represent significant difference with controls by covariance analysis; open symbols represent nonsignificance. The length of the lines represent the range of data for each group.

Fig. 3. Relation of Mn with DNA, RNA, and protein. Solid line represents the control. Dashed line represents the standard deviation. The equation to each normal relation is reported in the text. ○ ●: hypophysectomized rats. □ ■: calorie-restricted rats. Solid symbols represent significant difference with controls by covariance analysis; open symbols represent nonsignificance. The length of the lines represent the range of data for each group.

Table III. Covariance analysis

Source ¹	Control rats versus					
	Hypophysectomized rats			Calorie-restricted rats		
	df ²	Mean square	F	df	Mean square	F
Zn:DNA						
Reduction—fitting two lines	2	1,272.45	1.2106	2	10,471.14	9.5233***
Reduction—fitting two slopes				1	78.98	0.0718
Reduction—fitting two means				1	20,863.30	18.9749***
Pooled error	57	1,051.09		57	1,099.52	
SS about two lines						
Zn:RNA						
Reduction—fitting two lines	2	1,390.15	2.0739	2	2,325.47	3.2563
Pooled error	46	670.32		46	714.14	
SS about two lines						
Zn:protein						
Reduction—fitting two lines	2	3,034.12	6.2863**	2	1,192.80	2.3659
Reduction—fitting two slopes	1	2,061.04	4.2702			
Reduction—fitting two means	1	4,007.21	8.2024**			
Pooled error	58	482.66		58	504.16	
SS about two lines						

¹ See table I, footnotes 3 through 7.

² df = degrees of freedom.

** $P < 0.01$.

*** $P < 0.001$.

Manganese (table IV)

The Mn levels for given DNA or RNA values did not differ from the normal range in the hypophysectomized rat, but Mn was slightly reduced with respect to protein ($P < 0.025$). The levels were elevated above normal in rats subjected to calorie restriction ($P < 0.001$ for DNA or protein; $P < 0.005$ for RNA).

Ratios with DNA (table V)

Although the above comparisons were made using total amounts of DNA, RNA, protein, Zn, and Mn in liver, consideration was also given to the ratios with

DNA. In general, although the ratios were linearly related, the regression lines were not defined clearly. Correlation coefficients of 0.53–0.72 were found with proportionately high variances. Ratios present a statistical problem when both numerator and denominator are random variables that may be changing at different rates. Interpretation of the results from analysis of covariance using ratios can be difficult or misleading.

In the work presented here, however, it can be demonstrated that Zn and RNA retain a normal relation per unit DNA in the two abnormal situations con-

Table IV. Covariance analysis

Source ¹	Control rats versus					
	Hypophysectomized rats			Calorie-restricted rats		
	df ²	Mean square	F	df	Mean square	F
Mn:DNA						
Reduction—fitting two lines	2	2.3950	0.9659	2	140.59	45.7292***
Reduction—fitting two slopes				1	2.11	0.6869
Reduction—fitting two means				1	279.06	90.7714***
Pooled error	57	2.4796		57	3.07	
SS about two lines						
Mn:RNA						
Reduction—fitting two lines	2	1.0011	0.4307	2	14.18	5.1050**
Reduction—fitting two slopes				1	3.44	1.2367
Reduction—fitting two means				1	24.93	8.9732**
Pooled error	46	2.3244		46	2.78	
SS about two lines						
Mn:protein						
Reduction—fitting two lines	2	8.5518	4.7868*	2	43.09	18.8867***
Reduction—fitting two slopes	1	1.5011	0.8420	1	1.57	0.6877
Reduction—fitting two means	1	15.6024	8.7516**	1	84.61	37.0857***
Pooled error	58	1.7828		58	2.28	
SS about two lines						

¹ See table I, footnotes 3 through 7.

² df = degrees of freedom.

* $P < 0.025$.

** $P < 0.01$.

*** $P < 0.001$.

sidered (fig. 4). The three groups, therefore, were combined, as before, to calculate the following equation:

$$\frac{\text{Zn}}{\text{DNA}} = 3.951 + 2.001 \frac{\text{RNA}}{\text{DNA}}$$

where $N = 66$ and variance = 0.6524. The relation of Mn/DNA to RNA/DNA are included in table V for comparison.

Discussion

Data from several studies support a possible role of Zn in protein synthesis [12, 13, 18]; however, as pointed out by Fox and HARRISON [8], there have been no definite studies in animals. The present study demonstrates that liver RNA content follows liver Zn content over a variety of experimental conditions that are known to affect the subsequent synthesis of protein. The implication is that Zn has a primary role in protein synthesis and work by other investigators in lower forms of life supports this thesis. Moreover, a close relation is suspected between Zn and RNA for unicellular organisms and for plants [12, 24–26].

ALTMANN *et al.* [1], in studies with *Chorella pyrenoidosa*, showed an approximate but parallel increase of Zn and messenger RNA (mRNA), suggesting an influence of Zn ions on RNA synthesis. The growth of *Euglena*

Fig. 4. Solid line represents the relation of Zn/DNA to RNA/DNA calculated for control and experimental groups. Dashed lines represent standard deviation. Δ : control rats. $\circ$: hypophysectomized rats. $\square$: calorie-restricted rats. Open symbols represent no significant difference with controls by covariance analysis. The length of the lines represent the range of data for each group.

Table V. Covariance analysis

Source ¹	Control rats versus					
	Hypophysectomized rats			Calorie-restricted rats		
	df ²	Mean square	F	df	Mean square	F
Zn/DNA:RNA/DNA						
Reduction—fitting two lines	2	1.2073	0.6908	2	4.1831	2.3767
Pooled error	45	1.7477		45	1.7600	
SS about two lines						
Mn/DNA:RNA/DNA						
Reduction—fitting two lines	2	0.0040	0.9417	2	0.1478	26.6450***
Reduction—fitting two slopes				1	0.0069	1.2381
Reduction—fitting two means				1	0.2887	52.0519***
Pooled error	45	0.0043		45	0.0055	
SS about two lines						

¹ See table I, footnotes 3 through 7.

² df = degrees of freedom.

*** $P < 0.001$.

gracilis is proportional to the Zn concentration within the cell. In zinc-deficient *Euglena*, the earliest metabolic 'lesion' is a failure of RNA formation followed by interference with protein and DNA synthesis [19, 21].

Ribonucleic acid is capable of binding significant quantities of metal ions. WACKER and VALLEE [22] have made systematic studies of nucleic acid from diverse sources and have demonstrated that relatively large amounts of metals were firmly bound to these nucleic acids. It has been suggested that these metals have a role in determining and stabilizing the configuration of these nucleic acids and in maintaining their biological activity [6, 9, 16, 20]. Commercial RNA samples were reported to contain metals that interfered with both the assay of ribonuclease and ribonuclease inhibitor [20, 27]. Such metals were identified as Zn and Cu [27].

Though the present investigation concerns Zn and Mn, it is clear that other trace metals are important. Relation with nucleic acids may prove informative. For example, when calories are restricted, does the change in Mn within the liver cell reflect a change in mitochondrial mass as suggested by others [7]?

Until recently the main emphasis concerning the role of Zn in cell growth had fallen on the Zn-sensitive enzymes. Growth retardation that occurred because of Zn deprivation had been explained as the result of altered activity of these enzymes [17]. Whatever the role of Zn in growth, it would not appear to be a simple influence on Zn—enzyme interaction. When Zn-deficient *Neurospora* were repleted with Zn, alcohol dehydrogenase (a Zn metalloenzyme) activity increased appreciably but a source of nitrogen was required—as though protein synthesis must have occurred concomitantly [18]. WOOD and SIBLY [28] found that the activity of carbonic anhydrase (a Zn metalloenzyme) in oat leaves was reduced by a deficiency of Zn, the inhibition being related to the blocking of protein synthesis and not to a lack of sufficient Zn to activate the enzyme. KESSLER [11] demonstrated that the ribonuclease activity of apple leaves increased sharply with a decreased Zn content below 15 ppm. Studies of Zn deficiency in *Euglena* and other organisms indicated that the RNA molecule was curiously unstable [18]. Other studies demonstrated that RNA molecules containing Zn inhibited ribonuclease activity [27]. It is possible that Zn is necessary to protect liver RNA from attack by nuclease.

Recent studies of MACAPINLAC *et al.* [14] using the testes of Zn-deficient rats demonstrated a reduced total content of RNA and protein, but unaltered incorporation of adenosine-¹⁴C and leucine-¹⁴C into RNA and protein, respectively. They suggested that an increase in RNA and protein catabolism may occur in Zn deficiency rather than a decrease in synthesis.

Work on infants suffering from protein malnutrition (kwashiorkor) has demonstrated reduced RNA/DNA and Zn/DNA levels in muscle [4]; increased DNA, RNA, and Zn values occurred with rehabilitation. WARREN *et al.* [23], in a preliminary communication, record low values for Zn in the liver of children with marasmus and kwashiorkor. The present study demonstrates that in calorie-restricted rats (without protein deficiency) liver protein, RNA, Zn, and Mn, when related to total DNA, are all increased. Thus, these two types of restricted nutrition give rise to differential effects in tissues [10].

While Zn deficiency may exist in mammals as a definite entity, the hypothesis can be made that in protein deficiency, as with other situations, RNA content of the cell will fluctuate with the Zn content [3].

Clearly, further work must be done to elucidate the relation of Zn to RNA during other experimental conditions such as in simple Zn deficiency.

Summary

The relation of the metals, Zn and Mn, with DNA, RNA, and protein in the livers of normal, hypophysectomized, and calorie-restricted rats are examined. Statistical methods utilizing regression analysis and analysis of covariance demonstrate a strong and continuous relation between Zn and RNA. The biological implications of these findings are discussed.

References and Notes

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