Pediat. Res. *3:* 433–440 (1969)

Growth retardation
hypophysectomy
inanition
magnesium
manganese
zinc

Changes in Enzymes (GOT and GDH) and Metals (Zn, Mn, and Mg) in Liver of Rats During Endocrine Imbalance and Caloric Restriction

DONALD B. CHEEK[38] and JOAN E. GRAYSTONE

The Division of Growth, The Children's Medical and Surgical Center,
Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Extract

Restriction of essential trace metals in the diet interferes with growth. In this study the converse conditions pertained. Livers from calorie-restricted and hypophysectomized rats (with or without hormone treatment) were analyzed for protein, ribonucleic acid (RNA), deoxyribonucleic acid DNA), Mn, Zn, and Mg content and for enzyme activity of glutamic oxalic transaminase (GOT) and glutamic dehydrogenase (GDH). The data are expressed per unit of DNA in liver.

Hypophysectomized rats had reduced amounts of metal and enzyme activity in liver and also decreased protein and RNA:DNA ratios. Calorie-restricted rats with adequate protein intake showed opposite findings. GOT activity was not increased.

Injection of insulin or bovine growth hormone into hypophysectomized rats increased liver weight and protein and RNA content, but injection of growth hormone only increased DNA content. Injection of insulin increased Zn, Mn, and Mg content and GDH activity per unit of DNA. These results closely resembled the analyses in liver of a previously reported calorie-restricted group.

Injection of growth hormone decreased values per unit of DNA, but only significantly so for Zn, RNA, and protein.

Conjoint injection of growth hormone and epinephrine inhibited accretion of protein and reduced expected increments in DNA. The RNA:DNA ratio increased, however, and presumably, Mg and Zn were taken up by the liver cell. Sympathetic stimulation inhibited growth.

Activity of GDH in liver paralleled the Zn:DNA ratio, and the latter closely followed fluctuations in the RNA:DNA ratio. Activity of GOT, normally directed toward gluconeogenesis, increased in the absence of protein accretion.

Speculation

Zn and Mn play important intermediate roles in the action of hormones, particularly growth hormone, at the cellular level. Zn is closely retarded to both enzyme activity and RNA activity and to protein synthesis. The Mn:DNA ratio in liver reaches constancy at weaning, and departures from expected values at postweaning may represent changes in mitochondrial mass under abnormal conditions. The similarity in the analyses of liver of hypophysectomized rats treated with insulin and of calorie-restricted rats may indicate that insulin activity is more influential than is growth hormone activity during caloric restriction with adequate protein intake.

Introduction

Zinc, manganese, and magnesium play important roles in metabolic processes and are essential to life, fetal development, and growth [19, 20, 30]. These metals may be incorporated in metallo-proteins or may exist in a free ionic state within the cell [5]. Zinc may act as a cofactor for some of the dehydrogenase enzymes, and Mg is involved in many enzymatic reactions. The major fraction of Mn in the parenchymal cell of liver is present in mitochondria, and the remainder is found in microsomes and nucleus [2, 15]. Zinc is primarily found in cytoplasm [16].

Study of animals depleted of trace metals reveals limited information with respect to cell function. Additional information can be obtained by observing changes within cells during hormonal imbalance or under other experimental conditions. Glucocorticoids produce migration of Mn from liver to carcass, while growth inhibition resulting from Mn deficiency is said to be reversed by growth hormone [18]. Both hypophysectomy in rats and the injection of ACTH causes a loss of Zn from the prostate and adrenal glands [28]. Values for Zn, Cu, and Mn in the liver and muscle are typically correlated with immaturity in rats [6], while Mn per unit liver DNA is reduced with aging. Such changes are fully apparent only when DNA is used as a baseline [5, 6]. Pituitary dwarfs have lower than normal concentrations of Mn in muscle tissue [5].

The general understanding of the role of trace metals in human biology is still in the initial phase, and the customary approach is to monitor mammalian growth during a dietary intake of a trace metal below the essential level. It is important, however, to determine: 1. the level and content of trace metals in biological tissues when growth factors (hormones or nutrition) are modified; 2. those cellular components related to growth (nucleic acids or protein), which vary with concentration of trace metals; and 3. whether enzyme activity is related to changes in cell content of a specific trace metal.

During previous investigations [4, 14], samples of liver were weighed, frozen, and stored at −20°. The study dealt with the effects of growth hormone, insulin, or growth hormone and epinephrine on hypophysectomized rats. Control rats were pair fed to the caloric intake of hypophysectomized rats. Hypophysectomized rats had a reduction in liver DNA content for age and in RNA content per cell. Injection of either growth hormone or insulin for eleven days during an equal caloric intake caused increased liver weight and protein and RNA content. New DNA units were produced only by injection of growth hormone, and compared with normal liver, the protein:DNA ratio was decreased. Injection of insulin caused this ratio to be markedly increased above that found in normal age mates. Thus, in the latter instance, an increase in liver cell size was predicted, since polyploidy does not increase in the absence of growth hormone [11, 17]. Injection of long-acting epinephrine (Susphrine®) conjointly with growth hormone slowed DNA content incrementally and accumulation of protein in the liver, but increased the RNA:DNA ratio. Caloric restriction in the intact rat slowed increments of DNA in liver, but increased the RNA:DNA ratio. The augmented protein:DNA ratio was of borderline significance.

This study is concerned with the determination of the liver content of the metals Zn, Mn and Mg, and the enzymatic assay of glutamic dehydrogenase (GDH) and glutamic oxalic transaminase (GOT). Expression per unit of DNA has been chosen, since this value reflects the situation within the liver cell [5, 6, 12, 13].

Methods

The metals Mg, Zn, and Mn were measured by applying atomic absorption spectroscopy to an acid extract of liver following dehydration and reduction to an ash at 500° in a muffle furnace, as previously described [6]. Enzyme activity in the liver was measured by using the following procedure:

Approximately 40 mg of liver was homogenized in 1 ml of Tris-HCl buffer (pH 7.6) at 4°. Determinations were made on the clear supernatant obtained after centrifugation at 15,000 rpm for 30 min.

GDH activity was assessed by DPN formed at an incubation of a 10 μl aliquot of liver extract with 100 μl of a reagent containing DPNH (1.28 μM), γ-ketoglutarate (0.5 mM), nicotinamide (0.1 mM), ammonium chloride (0.75 mM), and crystalline bovine albumin (2.5 mg) in 5 ml of Tris-HCl buffer at pH 7.6. The reaction was allowed to proceed for 30 min at 38°. The sample was then placed in an ice bath, and 20 μl of 1 N HCl was added to destroy the DPNH and to stop the reaction. The DPN was measured by the method of KAPLAN *et al.* [22], originally described by LOWRY *et al.* [24]. A 10 μl aliquot of the mixture was added to 200 μl of 6.6 N NaOH, mixed thoroughly, and allowed to stand at room temperature for one hour. The sample was then diluted with 2 ml of deionized water. Blanks and standard solutions of DPN were determined concomitantly. Fluorescence was measured in a Zeiss spectrofluorometer against a quinine working standard, using a Xenon arc lamp as the light source. The uncorrected wave lengths were 365 mμ excitation and 470 mμ fluorescence.

GOT activity was measured by coupling the transaminase reaction with malic dehydrogenase [23]. The DPN formed is equivalent to the transaminase activ-

ity. 10 μl aliquots of the liver extract with substrate reagent were incubated as described for GDH, and here the incubation time was one hour. The substrate reagent was prepared immediately. DPNH (1.28 μM) and malic dehydrogenase solution (0.2 mg enzyme protein) were added to 2 ml of a solution containing aspartate (100 mM), γ-ketoglutarate (20 mM), nicotinamide (20 mM), and bovine albumin (0.5 g) in Tris-HCl buffer at pH 7.6.

The methods used for measuring protein, nucleic acids, and water content have been described previously [14]. Student's t test was used to determine the degree of statistical significance between grouped data.

Experimental Plan

The detailed design of the experiments carried out for the various groups of rats has been described [4, 14]. Briefly, normal male rats of Sprague Dawley strain [33] were used. These were hypophysectomized prior to shipment at 21 days of age. At 23 days of age, the rats were placed in individual metabolic cages and fed a diet of Purina chow (23 % protein). Both untreated rats and rats that received hormones by subcutaneous injections were offered 5 % sucrose to drink; normal rats fed an *ad libitum* diet were given tap water. Body weight and food and fluid intake were monitored daily.

At 38 days of age, the body weights of the hypophysectomized rats had become constant. The rats were then divided randomly into five groups. The first group was killed, the second left untreated, the third received bovine growth hormone [34] (250 μg/rat/24 h), the fourth received PZ insulin injections in increasing doses from 0.4 to 1.8 units [29], and the fifth group received growth hormone (250 μg/rat/24 h) plus injections of long-acting epinephrine in increasing amounts (2.5 to 20 μg). The experimental conditions were maintained for 11 days, at which time the rats were killed. From the 23rd to the 49th day those rats pair fed (per 100 g body weight) to untreated hypophysectomised rats received only 60 % of a normal intake and are designated 'calorie restricted'. A subgroup of these rats was killed at 38 days of age. Control rats receiving an *ad libitum* chow diet were killed at intervals from 26 to 49 days of age.

Caloric intake of the groups receiving either insulin or growth hormone was the same as that of the untreated hypophysectomized rats, while that of the group receiving growth hormone and epinephrine was higher.

Results

Values for concentrations of DNA, Zn, Mn, and Mg, reported per unit of fresh liver tissue, and the ratios of the metals, protein, and RNA per unit of DNA are

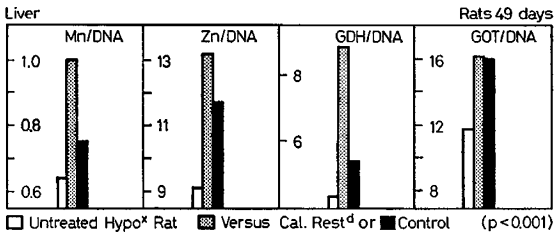

Fig. 1. The livers of hypophysectomized rats are compared with calorie restricted rats or control rats. Note that the Mn:DNA (μg/mg) and the Zn:DNA (μg/mg) of the liver are reduced in hypophysectomized rats and elevated in rats subjected to calorie restriction. A similar situation pertains for glutamic dehydrogenase (GDH). In the liver of hypophysectomized rat there is a decrease in the activity of glutamic oxalic transaminase (GOT).

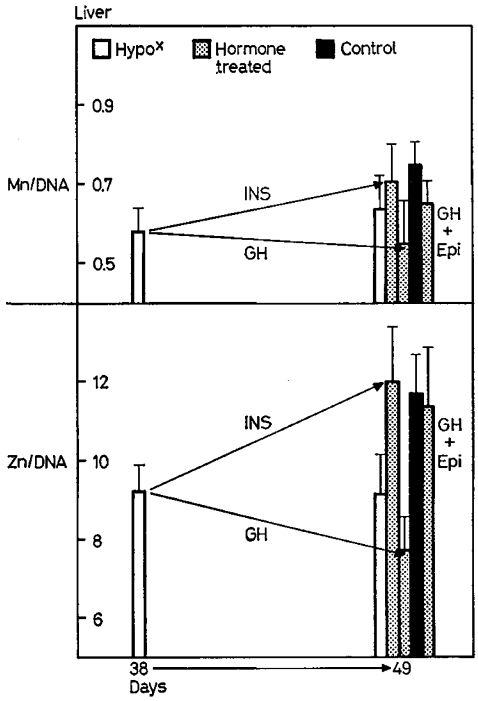

Fig. 2. The effect on the liver of giving growth hormone, insulin, or growth hormone and epinephrine conjointly, from 38 to 49 days to hypophysectomized rats is shown with respect to change in the Mn:DNA and Zn:DNA (μg/mg). The standard deviations (one tailed) are shown also. Note that insulin causes increases in these ratios and growth hormone decreases. The combination of epinephrine and growth hormone increased the Zn:DNA ratio.

Table I. Liver analyses

		Age, days	Body wt, g	Liver wt, g	DNA (mg/g) fresh	Zn (μg/g) fresh	Mn (μg/g) fresh	Mg (μg/g) fresh	Protein/ DNA	Zn/ DNA	Mn/ DNA	Mg/ DNA	RNA/ DNA, mg/mg
										μg/mg			
I. a) Normal	Mean	26	73.2	2.92	3.72	39.82	2.85	212.23	52.14	10.72	0.77	61.35	3.96
	S.D.		3.4	0.25	0.227	4.56	0.174	7.66	2.54	1.21	0.043	12.16	0.23
	n		7	7	7	7	7	7	7	7	7	8	7
b) Normal	Mean	28	81.6	4.06	3.84	37.48	2.62	215.63	48.70	9.76	0.68	56.41	
	S.D.		8.7	0.56	0.317	4.88	0.221	5.59	2.28	1.27	0.0575	4.14	
	n		6	6	6	6	6	6	6	6	6	6	
c) Normal	Mean	35	125.0	6.02	2.89	32.13	2.15	194.24	59.01	11.11	0.75	67.28	3.79
	S.D.		7.1	0.46	0.166	3.86	0.196	11.79	3.11	1.22	0.0579	4.32	0.51
	n		6	6	6	6	6	6	6	6	6	6	5
d) Normal	Mean	38	146.7	6.25	3.07	41.03	2.38	210.89	58.57	13.64	0.78	68.52	3.89
	S.D.		6.2	0.48	0.308	2.69	0.233	13.37	5.38	1.68	0.058	6.54	0.68
	n		6	6	5	6	6	6	5	5	5	5	5
II. Hypophysectomized	Mean	38	74.1	2.50	4.21	38.46	2.45	198.73	46.22	9.19	0.59	47.73	2.43
	S.D.		2.6	0.28	0.566	3.79	0.197	12.15	1.86	0.59	0.065	4.47	0.40
	n		8	8	8	8	8	8	8	8	8	8	8
III. Intact pairfed to hypo-	Mean	38	119.4	4.17	3.26	42.81	3.25	234.47	59.76	13.24	1.01	72.72	4.19
physectomized (calorie-	S.D.		3.5	0.43	0.449	3.61	0.269	16.53	8.46	0.98	0.12	7.48	0.65
restricted)	n		9	9	9	9	9	9	9	9	9	9	9
I. e) Normal	Mean	49	224.0	9.07	3.31	38.64	2.46	190.71	61.54	11.70	0.75	57.83	3.70
	S.D.		8.9	1.08	0.304	3.25	0.143	12.15	5.01	0.97	0.058	5.11	0.37
	n		7	7	7	7	7	7	7	7	7	7	7
V. Intact pairfed to hypo-	Mean	49	186.0	7.88	2.54	33.15	2.50	191.56	66.87	13.17	1.00	75.55	4.60
physectomized (calorie-	S.D.		6.9	0.39	0.177	1.81	0.256	18.72	4.58	0.96	0.11	6.73	0.27
restricted)	n		8	8	8	8	8	8	8	8	8	8	8
VI. Hypophysectomized	Mean	49	77.9	3.12	3.68	33.30	2.35	172.97	56.32	9.11	0.64	47.36	2.71
	S.D.		4.5	0.36	0.393	3.38	0.207	9.72	3.96	0.95	0.086	4.14	0.28
	n		9	9	9	9	9	9	9	9	9	9	9
VII. Hypophysectomized	Mean	49	105.6	4.61	4.08	31.19	2.22	191.56	51.70	7.68	0.55	47.09	2.66
+growth hormone	S.D.		3.5	0.40	0.437	3.21	0.307	14.71	3.55	0.82	0.12	2.89	0.18
	n		8	8	8	8	8	8	8	8	8	8	8
VIII. Hypophysectomized	Mean	49	91.9	4.45	3.05	37.08	2.16	205.30	68.66	11.95	0.71	69.57	3.50
+insulin	S.D.		4.6	0.76	0.593	5.01	0.241	36.71	5.81	1.42	0.11	14.79	0.43
	n		8	8	8	8	7	8	8	8	7	7	8
IX. Hypophysectomized	Mean	49	105.6	3.83	3.58	40.26	2.28	235.55	46.61	11.34	0.64	66.46	3.42
+growth hormone	S.D.		2.45	0.40	0.434	3.28	0.226	6.95	3.15	1.345	0.051	7.35	0.38
+epinephrine	n		7	7	7	7	7	7	7	7	7	7	7

The group numbers for the rats are similar to those used previously [4, 14].

shown in table I. The activity of GDH and GOT in relation to protein and DNA content in liver is shown in table II.

Calorie-restricted Rats

This group had higher values per unit of DNA for Zn ($p < 0.01$), for Mn and Mg ($p < 0.001$), and for GDH ($p < 0.001$) than did normal rats (fig. 1). For GOT, values were the same. The RNA:DNA ratio was high compared with that of normal rats ($p < 0.001$); however, the protein:DNA ratio was of borderline significance.

Hypophysectomized Rats

At 38 or 49 days of age, untreated hypophysectomized rats had low values of protein, RNA, Zn, Mn, and Mg per unit of DNA, compared with values in control rats of the same age ($p < 0.001$) (table I and figs. 1 and 2). At 38 days of age, values for GOT ($p < 0.001$) and GDH ($p < 0.01$) in liver were also reduced per unit of DNA (table II). At 49 days of age, values for GOT showed only a borderline reduction ($p < 0.05$). These changes would not be so apparent if data had been reported per unit of fresh tissue or per unit of protein.

At 49 days of age, the body weights of untreated hypophysectomized rats and those of 26-day-old rats were not statistically different. The ratios of Zn:DNA ($p < 0.05$), Mn:DNA ($p < 0.01$), and Mg:DNA ($p < 0.01$) in liver of hypophysectomized rats were decreased, although the ratio of protein:DNA was slightly increased ($p < 0.05$). Comparison of enzyme activities per unit of body weight revealed no apparent differences.

Hypophysectomized Rats Receiving Growth Hormone or Insulin

Injection of growth hormone increased the content of DNA, RNA, and protein in liver of the hypophysectomized rats and reduced the ratios of RNA:DNA and protein:DNA ($p < 0.001$). Compared with untreated hypophysectomized 49-day-old rats, the values for Zn, Mn, Mg, and GDH also fell (figs. 1, 2, and 3); however, the difference was significant only in the value for Zn ($p < 0.01$).

Injection of insulin during the same period increased

Table II. Liver enzyme activity[1]

		Age, days	Glutamic acid dehydrogenase		Glutamic oxalacetic transaminase	
			μM/mg DNA/h	μM/mg protein/h	μM/mg DNA/h	μM/mg protein/h
I. a) Normal	Mean	26	5.27	0.10	10.11	0.20
	S.D.		1.42	0.03	1.56	0.03
d) Normal	Mean	38	5.61	0.12	12.73	0.28
	S.D.		0.95	0.01	1.19	0.07
II. Hypophysectomized	Mean	38	3.06	0.06	7.66	0.17
	S.D.		0.93	0.01	1.34	0.03
I. e) Normal	Mean	49	5.42	0.09	15.94	0.26
	S.D.		1.02	0.01	3.32	0.04
V. Intact pairfed to hypophysectomized (calorie-restricted)	Mean	49	8.92	0.13	16.16	0.24
	S.D.		1.03	0.01	4.18	0.05
VI. Hypophysectomized	Mean	49	4.26	0.08	11.80	0.21
	S.D.		1.52	0.02	1.55	0.03
VII. Hypophysectomized +growth hormone	Mean	49	2.59	0.05	9.62	0.18
	S.D.		1.55	0.03	3.53	0.06
VIII. Hypophysectomized +insulin	Mean	49	7.96	0.12	9.70	0.14
	S.D.		1.30	0.02	1.14	0.02
IX. Hypophysectomized +growth hormone +epinephrine	Mean	49	8.37	0.18	15.98	0.34
	S.D		2.22	0.04	4.20	0.07

The group numbers for the rats are similar to those used previously [4, 14].

[1] 6 rats/group.

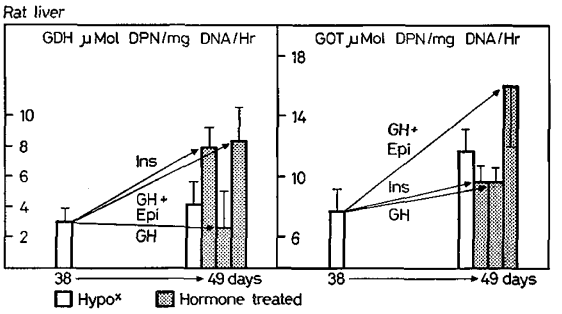

Fig. 3. The effect on the liver enzymes, glutamic dehydrogenase, and glutamic oxalic transaminase is shown when various hormones are given to hypophysectomized rats. Note that transaminase activity decreased when insulin or growth hormone were given (and when protein accretion was occuring). Dehydrogenase activity tended to follow the Zn:DNA ratio or the Zn:RNA ratio.

the content of RNA, Zn, Mn, Mg, and GDH per unit of DNA ($p < 0.001$). The ratio of protein:DNA was higher in animals receiving insulin than it was in any other group. Injection of both insulin and growth hormone tended to decrease transaminase activity in the liver, although the decrease was significant only after insulin administration ($p < 0.025$) (fig. 3).

Hypophysectomized Rats Receiving Growth Hormone and Epinephrine

Hypophysectomized rats had the same protein content in liver as hypophysectomized rats receiving growth hormone and epinephrine conjointly. In contrast, hypophysectomized rats receiving growth hormone alone had a markedly increased protein content in liver [4]. The additional injections of epinephrine and growth hormone did not change the content of Mn per unit of DNA, but Zn and Mg per unit of DNA increased significantly ($p < 0.001$). Activity of GDH also increased ($p < 0.001$), and although that for GOT was elevated, the increase was of borderline significance ($p < 0.05$).

Discussion

Absence of pituitary function reduces the amount of Zn, Mn, and Mg per unit of DNA in the liver of hypophysectomized rats in comparison with the amounts of these metals per unit of DNA present in normal rats of the same weight. This finding indicates a true loss of trace metal, not just immature levels [6]. Our previous study [6] showed that these trace metals, as well as Cu, were reduced in muscle as well as in liver tissue of hypophysectomized rats. Since thyroid ablation causes degeneration of acidophil cells in the pituitary and depletion of tissue trace metal [6], the question arises whether reductions of Zn, Mn, and Cu are related to deficiency of growth hormone *per se*. Unpublished data indicate that there is a loss of Zn and Mn in cerebral cells following hypophysectomy. Conversely, dietary restriction of these trace metals will inhibit growth in the intact rat [19, 20, 27]. These metals play an important intermediate role in the process of growth and probably in the actions of hormones at the cellular level. It has been shown that trivalent chromium is necessary for the proper action of insulin at the mitochondrial level within the liver cell [3].

Whether the reduced activity of liver enzymes in hypopituitarism, reported in this study and by others [21], is related to the loss of trace metal remains to be investigated. When GDH activity has been found to increase, however, so has the cofactor Zn. A rough linear relation exists between the two. PRASAD *et al.* [27] consider that growth inhibition caused by Zn deficiency results from interference in the activity of Zn-dependent enzymes.

In the present study, the finding that Zn, Mn, and Mg content and GDH activity increased per unit of DNA in liver with injection of insulin, but tended to decrease with injection of growth hormone, suggests that these cytoplasmic components fluctuate according to the ratios of protein:DNA or RNA:DNA. With respect to insulin, one would suspect enlarged cytoplasm with a diploid nucleus. Such a condition would be in contrast to the constant relation that exists in normal liver cells in which the cytoplasmic mass is proportional to the degree of polyploidy [12, 13].

Further reductions in trace metals per unit of DNA following injection of growth hormone were only significant for Zn. It is possible that some redistribution of new and existing cytoplasm occurs through establishment of a higher polyploid series (tetraploid or octaploid cells), with relatively less, but adequate, amounts of metal and cytoplasm available upon nit of DNA. The actual number of liver cells may not change. Under these circumstances, a large nucleus containing four or eight times as much DNA would be present; however, the cytoplasmic mass, probably in the state of potential expansion, would not increase at the same rate. Another interpretation may be that endogenous insulin is insufficient in relation to growth hormone. In the liver cell, the two hormones appear to act in different directions, growth hormone affecting DNA increase [1, 4, 8, 10] and insulin affecting protein accretion [4].

It has been reported that in rats subjected to caloric restriction, the DNA content in liver was not increased; however, the RNA:DNA ratio was excessively high

[14]. Activity of GDH and content of Zn, Mn, and Mg per unit of DNA were also increased. It is possible that by restricting calories but not protein, cellular activity may be concerned with protein accretion, and growth may occur by increments in cell size. The values in calorie-restricted rats were comparable with those of hypophysectomized rats treated with insulin. On the basis of previous investigations [4, 14] and the present study, the suggestion can be made that in caloric restriction without protein deprivation, the influence of insulin is greater than that of growth hormone.

The suggestion that changes in the concentration of trace metal and Mg follow the changes in the protein:DNA ratio was not substantiated by the results obtained by injecting epinephrine and growth hormone conjointly. Although the concentration of Mn remained unchanged in the liver cell, concentrations of Zn and Mg were markedly increased. Storage of adrenaline in granular vesicles of cells requires ATP and Mg, as well as Cu and Zn [9]. It is possible that in the present study, uptake of Mg and Zn was a manifestation of such storage [35]; or, perhaps, Mg and Zn followed the change in the RNA:DNA ratio, which was markedly elevated.

In table I, the RNA:DNA ratio found in normal and abnormal rats [4, 14] is plotted against the Zn:DNA ratio. The two ratios have a linear relation. Fluctuations in Zn appear to be more closely related to changes in cell RNA. RNA elevation is a precursor of protein synthesis [4, 31], and Zn may be equally involved.

In the present study, a fixed amount of Mn was found to be present per unit of DNA in liver of normal rats 26 to 49 days of age. In a previous study [6], it was found that this ratio was increasing at 21 days of age, although in muscle, the ratio was constant at 3, 5, and 8 weeks of age. If Mn is a measure of mitochondrial mass in the liver [2], this mass may reach constancy shortly after weaning. The value found for Mn was 0.74 μg per mg of DNA in liver, while the range of the RNA:DNA ratio was only 3.7 to 4.0 mg per mg of DNA from 26 to 49 days of age.

Hypophysectomized rats at 21 days of age had less than normal amounts of Mn and RNA per unit of DNA, while calorie-restricted rats had more. Thus, it is possible that the mitochondrial mass fluctuates in accordance with protein turnover in the cell.

Transaminases may divert amino acids into the carbohydrate cycle and stimulate gluconeogenesis. The injection of insulin into the hypophysectomized rat was associated with a reduced activity of GOT per unit of DNA. GDH activity, however, increased at a time when protein accretion was occurring. Perfusion of the liver in the presence of insulin has been shown to inhibit proteolysis [25]. Under such circumstances, one would anticipate reduced activity of the GOT assayed. Since growth hormone stimulates insulin production, a similar result might be expected. In contrast, epinephrine inhibits insulin release [26], and the finding of increased GOT activity, together with a failure of protein accretion in liver, is also compatible with our findings. In our previous study [14], intact rats with hyperphagia that were injected with insulin also had reduced GOT activity ($p < 0.001$), but there was no marked protein accretion.

Injection of insulin into hypophysectomized rats resulted in a higher Mg:protein ratio in liver than that in liver of controls ($p < 0.02$). Since insulin is known to increase transport of K across the cell membrane [32], the same situation may hold for Mg.

Summary

The liver of normal rats, calorie-restricted rats, and hypophysectomized rats (maintained with and without hormone administration) were analyzed for Zn, Mn, and Mg content and for glutamic oxalic transaminase or glutamic dehydrogenase activity. The possible significance of data analysis in relation to changes in DNA, RNA, and protein in liver were discussed.

References and Notes

1. Beach, R.K. and Kostoyo, J.L.: Effect of growth hormone on the DNA content of muscles of young hypophysectomized rats. Endocrinology *82:* 882 (1968).
2. Borg, D.G. and Cotzias, G.C.: Manganese metabolism in man: rapid exchange of Mn^{56} with tissue as demonstrated by blood clearance and liver uptake. J. clin. Invest. *37:* 1269 (1958).
3. Campbell, W.J. and Mertz, W.: Interaction of insulin and chromium (III) on mitochondrial swelling. Amer. J. Physiol. *204:* 1028 (1963).
4. Cheek, D.B. and Graystone, J.E.: The action of insulin, growth hormone, and epinephrine on cell growth in liver, muscle, and cerebrum of the hypophysectomized rat. Pediat. Res. *3:* 77–88 (1969).
5. Cheek, D.B.; Reba, R.C. and Woodward, K.: Cell growth and the possible role of trace minerals; in: D.B. Cheek Human growth (Lea and Febiger, Philadelphia, Pa. 1968).
6. Cheek, D.B.; Powell, G.K.; Reba, R. and Feldman, M.: Manganese, copper and zinc in rat muscle and liver cells and in thyroid and pituitary insufficiency. Bull. Johns Hopk. Hosp. *118:* 338 (1966).

7. CHEEK, D.B. and MELLITS, E.D.: Unpublished data.
8. CHEEK, D.B.; POWELL, G.K. and SCOTT, R.E.: Growth of muscle cells (size and number) and liver DNA in rats and Snell Smith mice with insufficient pituitary, thyroid or testicular function. Bull. Johns Hopk. Hosp. *117:* 306 (1965).
9. COLBURN, R.W. and MAAS, J.W.: Adenosine triphosphate-metal-norepinephrine ternary complexes and catecholamine binding. Nature, Lond. *208:* 37 (1965).
10. DAUGHADAY, W.H. and REEDER, C.: Synchronous activation of DNA synthesis in hypophysectomized rat cartilage by growth hormone. J. Lab. clin. Med. *68:* 357 (1966).
11. DI STEFANO, H.S. and DIERMEIER, H.F.: Effects of hypophysectomy and growth hormone on ploidy distribution and mitotic activity of rat liver. Proc. Soc. exp. Biol. Med. *92:* 590 (1956).
12. EPSTEIN, C.J.: Cell size, nuclear content, and the development of polyploidy in the mammalian liver. Proc. nat. Acad. Sci. *57:* 327 (1967).
13. EPSTEIN, C.J.; MOSES, H.L.; EPSTEIN, L.B. and GARRISON, N.M.: A structural analysis of hepatomegaly induced by a hormone-secreting tumor. Exp. molec. Path. *7:* 304 (1967).
14. GRAYSTONE, J.E. and CHEEK, D.B.: The effects of reduced calorie intake and increased calorie intake (insulin induced) on the cell growth of muscle, liver, and cerebrum, and on skeletal collagen in the post weanling rat. Pediat. Res. *3:* 66–76 (1969).
15. GILBERT, I.G.F. and RADLEY, J.M.: Manganese and radioactive manganese in liver cell nuclei. Biochim. biophys. Acta. *79:* 575 (1964).
16. HARRISON, M.F.: Composition of the liver cell. Biochem. J. *55:* 203 (1953).
17. HELWIG-LARSON, H.F.: Nuclear class series: studies on frequency distribution or nuclear sizes and quantitative significance of formation of nuclear class series for growth of organs in mice with special reference to the influence of pituitary growth hormone. Acta. path. microbiol. scand. Suppl. 92 (1952).
18. HUGHES, E.R. and COTZIAS, G.C.: Growth, hormones and manganese. Amer. J. Dis. Child. *102:* 570 (1961).
19. HURLEY, L.S.: Studies on nutritional factors in mammalian development. J. Nutr. Suppl. 2 ad *91:* 27 (1967).
20. HURLEY, L.S. and SWENERTON, H.: Congenital malformations resulting from zinc deficiency in rats. Proc. Soc. exp. Biol. Med. *123:* 692 (1966).
21. JACOBSOHN, D.; LARSSON, S. and NORGREN, A.: Enzyme activities in various organs of hypophysectomized rats and rabbits. Acta physiol. scand. *63:* 271 (1965).
22. KAPLAN, N.O.; COLOWICK, S.P. and BARNES, C.C.: Effect of alkali on diphosphopyridine nucleotide. J. biol. Chem. *191:* 461 (1951).
23. LAURSEN, T. and ESPERSEN, G.: A fluorimetric method for measuring the activity in serum of the enzyme glutamic oxalacetic transaminase. Scand. J. clin. Lab. Invest. *11:* 61 (1959).
24. LOWRY, O.H.; ROBERTS, N.R. and LEWIS, C.: The quantitative histochemistry of the retina. J. biol. Chem. *220:* 879 (1956).
25. MORTIMORE, G.E. and MONDON, C.E.: Inhibition of proteolysis by insulin in perfused rat liver. Fed. Proc. *27:* 495 (1968).
26. PORTE, D.: A receptor mechanism for the inhibition on insulin release by epinephrine in man. J. clin. Invest. *46:* 86 (1967).
27. PRASAD, A.S.; OBERLEAS, D.; WOLF, P. and HORWITZ, J.P.: Studies on zinc deficiency: changes in trace elements and enzyme activities in tissues of zinc-deficient rats. J. clin. Invest. *46:* 4 (1967).
28. RUDZIK, A.D. and RIEDEL, B.E.: The effects of hypophysectomy and ACTH on zinc metabolism in the sex glands and adrenal of the male rat. Canad. J. Biochem. Physiol. *38:* 1003 (1960).
29. SALTER, J. and BEST, C.H.: Insulin as a growth hormone. Brit. med. J. *ii:* 354 (1953).
30. SCHROEDER, H.A.: Trace metals and chronic diseases. Adv. intern. Med. *8:* 259 (1956).
31. WAGLE, S.R.: The influence of growth hormone, cortisol and insulin on the incorporation of amino acids into protein. Arch. biochem. biophys. *102:* 373 (1963).
32. ZIERLER, K.L.: Increase in resting membrane potential of skeletal muscle produced by insulin. Science *126:* 1067 (1957).
33. Hormone Assay Laboratories, Chicago.
34. This hormone was supplied by the endocrine study section of NIH, batch number NIH-GH-B12 with a potency of 0.97 USP units/mg.
35. We find that this increased uptake of Zn and Mg also occurs in the liver of the intact rat given the same dose schedule of epinephrine (without growth hormone) for the same period of time.
36. This work was supported by grant HD 00126-05 from the National Institute of Child Health and Human Development, United States Public Health Service.
37. The authors acknowledge the technical assistance of RACHEL SCOTT.
38. Requests for reprints should be addressed to: DONALD B. CHEEK, M.D., Department of Pediatrics, Johns Hopkins Hospital, Baltimore, Md. 21205 (USA).