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Changes in Cellular Composition during Magnesium Deficiency

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1. Mg deficiency increased the water content of the liver, kidney, heart and thigh muscle in the rat and decreased the proportion of nitrogen in the dry matter of the same tissues. Changes in the concentration of metals also occurred. 2. Cellular fractionation indicated that the Mg and K depletion in liver occurred primarily in the heavy-mitochondrial and microsomal fractions respectively. The calcification of liver and kidney was due to preferential deposition of Ca in the heavy-mitochondrial fraction. 3. The proportion of cellular nitrogen present in the heavy-mitochondrial and microsomal fractions was markedly decreased in the liver and kidney of the Mg-deficient rats, and the proportion in the supernatant fraction increased. 4. Electron microscopy revealed that the cross-sectional areas of liver cells and all their mitochondria were decreased in Mg deficiency. The number of mitochondria per cell was decreased even more severely and the average area of a mitochondrion was greater in deficient rats than in control animals. 5. The significance of these observations is discussed in relation to the location of the primary metabolic disturbance during Mg deficiency.

Mg is an essential constituent of living organisms and deficiency has been shown to cause illness and death in animals of several species. Although Mg activates many enzymes *in vitro* (Wacker, 1969), comparatively few metabolic disturbances have been unequivocally established during its deficiency *in vivo* (Heaton, 1973). This may be partly because the Mg concentration in soft tissues is well maintained during dietary depletion, but some cellular loss of the metal does occur in severe deficiency and may be accompanied by a loss of K (MacIntyre & Davidsson, 1958; Martindale & Heaton, 1964). Little, however, is known about the secondary effects of the deficiency on other cellular constituents and the present investigation, where chemical analysis of subcellular fractions has been combined with examination of sections from the same organs by electron microscopy, was undertaken to examine this in more detail in the rat.

Experimental

Animal management

Two experiments were performed with male Wistar rats weighing about 100g. They were randomly divided into Mg-deficient and control groups, and pair-fed with the appropriate diets for 20 days with an automatic feeding apparatus (Loveless *et al.*, 1972) to prevent any difference in feeding pattern. Distilled water was provided *ad libitum*. The synthetic diets were prepared by mixing (g/kg): casein, 200; sucrose, 660; arachis oil, 80; cod-liver oil, 20; salt mixture, 40 and purified vitamins as described previously (Heaton & Anderson, 1965). They were identical in composition, apart from the incorporation of $MgCl_2$ in the control diet, and the Mg content determined by analysis was 1.0mg/kg (deficient) and 790mg/kg (control).

At the end of an experiment, rats were killed by exsanguination from the heart under diethyl ether anaesthesia and the heparinized plasma was separated immediately. The organs required for analysis in Expt. 1 were removed and stored individually in plastic vials at −25°C. In Expt. 2, when liver and kidneys were required for cellular fractionation, they were perfused through the aorta with 40ml of cold 0.25M-sucrose before removal from the body.

Cellular fractionation

The liver and kidneys from each rat in Expt. 2 were immediately homogenized with ice-cold 0.25M-sucrose in a Potter–Elvehjem homogenizer fitted with a Teflon pestle to give 25% (w/v) homogenates. The homogenates were separated into nuclear, heavy-mitochondrial, light-mitochondrial, microsomal and supernatant fractions by differential centrifugation under the conditions specified in Table 1. These were adopted, after trials with various centrifugal forces, to give optimum separations of nuclear and mitochondrial fractions as judged by microscopical examination and the determination of DNA content. Each fraction was washed once with one-half the original volume of sucrose solution and resuspended in distilled water for analysis.

Analytical methods

The water content of tissues was determined by drying at 105°C for 16h. The dry tissue was then ground to a fine powder and portions of this were used for further analysis. Lipid was determined by measuring the loss in weight when extracted with diethyl ether for 7h in micro-Soxhlet apparatus. Nitrogen was estimated by the micro-Kjeldahl method in samples of tissue powder and subcellular fractions; replicate analyses on tissues and trials with standard compounds showed that the procedure had a reproducibility and accuracy of 100±2%. Other portions of the same specimens were dry-ashed for mineral analysis by heating in a muffle furnace at 500°C for 24h and the ash was dissolved in 2M-HCl. Plasma was deproteinized with 10% (w/v) trichloroacetic acid before analysis.

Mg and Ca were determined by using a Unicam SP.90 atomic-absorption flame photometer, and Na and K were measured by emission analysis on the same instrument. All sample and standard solutions contained 0.1M-HCl, and for the determination of Ca, 2500mg/litre of Sr was also added (as $SrCl_2$) to prevent interference by other constituents of the samples. The chloride content of plasma and tissue homogenates was measured with an Aminco chloride titrator and the DNA present in tissue homogenates and subcellular fractions was measured colorimetrically with indole after extraction with trichloroacetic acid (Hubbard *et al.*, 1970).

Electron microscopy

The central lobe of the liver from rats in Expt. 2 was cut into small blocks which were immediately fixed for 5h with cold 4% (v/v) glutaraldehyde in 0.1M-sodium cacodylate buffer, pH7.0, containing 0.25M-sucrose. After two washes in cacodylate–sucrose buffer the blocks were stained overnight with 2% (w/v) OsO_4 in 0.1M-cacodylate buffer, pH7.0. The tissue was washed with the same buffer, dehydrated by solutions of increasing ethanol concentration, passed through propylene oxide and embedded in Epon 812 resin at 60°C. Sections of 50nm thickness were cut with an LKB Ultratome III and supported on carbon–Formvar-coated grids. Contrast was increased by staining with saturated ethanolic uranyl acetate and Reynolds lead citrate, and the sections were examined at 40kv in an AEI 801 electron microscope. Micrographs of whole cells were made on Rapidoline film (Agfa-Gevaert Ltd., Brentford, Middx., U.K.) and contrast was enhanced by processing in Pentelex X-ray developer (May and Baker Ltd., Dagenham, Essex, U.K.). Prints with a total magnification of 2560 diameters were made on hard paper. Each print was cut to the outline of the cell membrane and the nucleus was painted over with opaque white medium so the mitochondria appeared dark on a pale grey background.

The prints were individually presented to a Quantimet 720D television microdensitometer (Metals Research, Melbourn, Cambridge, U.K.) which was adjusted to record the cell area and the number and total area of mitochondria within the cell. A total of 50 cells were examined from each group of six Mg-deficient and control rats. No more than two cells were taken from any section, so at least four or five section of liver were studied from each rat.

Results

Gross chemical composition of tissues

A similar degree of Mg deficiency was produced in the two experiments and the mean body weight of the deficient and control rats increased to about 125 and 150g respectively. The plasma analyses from Expt. 1 indicate that the hypomagnesaemia was accompanied by hypercalcaemia and hypokalaemia (Table 2).

Analysis of various soft tissues from these rats revealed that the water contents of the liver, kidneys, heart and thigh muscle were all increased, and the proportion of N in the dry matter of the same tissues was consistently decreased in Mg-deficient rats compared with control animals (Table 3). The Mg concentration in the dry tissue was significantly decreased by about 25% only in liver and thigh muscle, but the Ca concentration was increased in all four tissues. Statistically significant K depletion was only observed in heart, but the liver showed a similar tendency. The presence of extracellular fluid

Table 1. *Conditions for separation of liver and kidney subcellular fractions by differential centrifugation in* 0.25M-*sucrose*

Fraction	Centrifugation time (min)	Centrifugal force ($g_{av.}$) Liver	Kidney
Nuclei	10	190	400
Heavy mitochondria	10	1000	1000
Light mitochondria	10	11500	11500
Microsomal fraction	60	150000	150000

Table 2. *Electrolyte concentrations in plasma of Mg-deficient and control rats*

Mean values±S.E.M., $n = 10$ for each group.

Metal	[Metal] (mg/litre of plasma) Control rats	Mg-deficient rats	P
Mg	20.4±0.2	6.3±0.1	<0.001
Ca	109±1	123±1	<0.001
K	199±1	183±1	<0.001
Na	3030±20	3040±10	>0.9

Table 3. *Composition of tissues from Mg-deficient and control rats*

Mean values ± S.E.M., $n = 12$ for each group. Water is expressed as mg/g fresh weight, all other constituents as mg/g dry weight of tissue. Values significantly different from control: $*P<0.05$; $**P<0.01$; $***P<0.001$.

Tissue	Group of rats	Water	Lipid	Nitrogen	Mg	Ca	K	Na
Liver	Control	708 ± 2	210 ± 12	124 ± 3	0.68 ± 0.02	0.12 ± 0.01	5.5 ± 1.0	1.7 ± 0.3
	Mg-deficient	719 ± 3**	194 ± 4	112 ± 3*	0.50 ± 0.02**	0.19 ± 0.01***	4.4 ± 0.8	1.8 ± 0.5
Kidneys	Control	717 ± 4	161 ± 3	107 ± 0.5	0.60 ± 0.01	0.30 ± 0.05	6.8 ± 0.5	1.5 ± 0.3
	Mg-deficient	737 ± 4**	157 ± 2	104 ± 0.2***	0.60 ± 0.03	0.69 ± 0.02***	6.4 ± 0.5	1.6 ± 0.2
Heart	Control	774 ± 2	207 ± 2	136 ± 0.3	0.77 ± 0.02	0.25 ± 0.01	6.9 ± 0.2	1.7 ± 0.2
	Mg-deficient	787 ± 2***	207 ± 2	126 ± 0.6***	0.74 ± 0.02	0.34 ± 0.01***	6.0 ± 0.1**	1.9 ± 0.3
Thigh muscle	Control	728 ± 4	253 ± 2	135 ± 1	0.95 ± 0.02	0.37 ± 0.04	4.7 ± 0.3	1.6 ± 0.2
	Mg-deficient	754 ± 4**	253 ± 3	117 ± 1***	0.68 ± 0.05***	0.53 ± 0.05*	5.0 ± 0.3	1.8 ± 0.2
Femur	Control				4.1 ± 0.6	171 ± 1	0.13 ± 0.01	5.0 ± 0.5
	Mg-deficient				1.7 ± 0.1***	184 ± 2**	0.13 ± 0.01	6.7 ± 0.2**

would contribute to the concentrations of Na and Ca found in the tissues, and the magnitude of this was investigated by measuring the chloride concentration in the plasma and in homogenates of liver and kidney. Assuming that all the chloride in tissue homogenates was of extracellular origin and that the metal/chloride ratio in interstitial fluid was the same as in plasma, this indicated that 6.2% of the Na and 0.6% of the Ca in liver was of extracellular origin; in kidney no more than 1% of either metal could be from extracellular fluid. Although this method gives only an approximate estimation of contamination by extracellular fluid, it indicates that the results observed were due predominantly to changes in the concentration of intracellular components.

The Mg concentration in the femur of deficient rats was only 41% of that in controls, which confirms the severity of the deficiency, and the concentrations of Ca and Na were increased.

Subcellular distribution of Mg, K, Na, Ca and N in liver and kidney

The amount of each metal in the subcellular fractions from rats in Expt. 2 was related to the nitrogen content of the fraction as an indication of its metabolic mass. The severity of the depletion was very similar to that obtained in Expt. 1, as judged by the lowered magnesium concentrations in the plasma and total liver homogenate (Table 4). Only the heavy mitochondria from the liver of deficient rats showed a significant fall in Mg concentration compared with control animals and the Mg concentration in nuclei rose. A similar rise in Mg concentration was observed in the nuclear fraction from the kidney of deficient animals, although there was no significant change in the total kidney homogenate. The elevated Ca concentration found in both organs from Mg-deficient rats was largely due to preferential deposition in the heavy-mitochondrial fraction, and the K depletion observed in the total liver homogenate was primarily the result of loss from the microsomal fraction, but the concentration in the nuclei of deficient rats again increased.

Examination of the distribution of individual substances between different compartments of the cell revealed another disturbance during Mg deficiency. The proportion of cellular nitrogen present in the heavy-mitochondrial and microsomal fractions was markedly decreased in both liver and kidney from Mg-deficient rats, and the proportion present in the supernatant fraction was increased (Table 5). This difference in nitrogen distribution, combined with the changes in concentration of individual metals, meant that the amounts of Mg and K were both decreased in the heavy-mitochondrial fraction of liver and kidney from deficient

Table 4. *Concentrations of Mg, K, Na and Ca in subcellular fractions of liver and kidney from Mg-deficient and control rats*

Mean values ±S.E.M., $n = 6$ for each group. Values significantly different from control: $*P<0.05$; $**P<0.01$; $***P<0.001$.

Tissue	Metal	Group of rats	Concentration in fraction (mg/g of nitrogen)				
			Total homogenate	Nuclei	Heavy mitochondria	Light mitochondria	Microsomal fraction	Supernatant
Liver	Mg	Control	5.6±0.3	6.4±0.2	3.7±0.1	2.6±0.2	9.1±0.2	5.1±0.3
		Mg-deficient	4.5±0.2*	7.9±0.2***	3.4±0.1*	2.2±0.2	9.0±0.1	4.9±0.2
	K	Control	44.4±1.9	20.1±0.3	4.9±0.4	8.1±0.3	20.7±0.5	165±2
		Mg-deficient	37.9±1.1*	22.2±0.6*	3.8±0.7	7.9±0.2	15.4±0.6**	167±3
	Na	Control	14.9±1.3	7.1±1.0	2.0±0.3	2.2±0.2	5.7±0.2	58.8±1.2
		Mg-deficient	15.9±1.0	7.3±0.8	2.3±0.2	2.0±0.3	6.0±0.3	60.5±1.2
	Ca	Control	0.97±0.1	1.7±0.3	0.80±0.05	0.79±0.06	3.4±0.2	1.7±0.1
		Mg-deficient	1.70±0.2**	1.9±0.1	0.96±0.03*	0.81±0.09	3.3±0.3	1.6±0.1
Kidneys	Mg	Control	5.5±0.5	6.0±0.1	2.7±0.6	2.1±0.1	7.5±0.2	6.8±0.1
		Mg-deficient	5.7±0.8	7.9±0.2***	2.5±0.4	1.9±0.1	7.5±0.2	6.6±0.2
	K	Control	61.9±1.3	73.1±2.9	9.0±0.8	10.1±0.6	11.2±0.6	195±4
		Mg-deficient	62.0±1.1	69.3±4.1	9.6±0.7	9.9±0.7	12.0±0.3	199±5
	Na	Control	16.1±0.5	17.3±1.2	4.6±0.6	5.9±0.2	7.1±0.3	43.9±0.9
		Mg-deficient	16.4±0.8	18.0±0.9	4.4±0.6	6.1±0.3	6.9±0.2	42.6±0.9
	Ca	Control	2.8±0.2	5.9±0.3	2.9±0.1	2.9±0.3	7.6±0.4	5.0±0.1
		Mg-deficient	6.7±0.2***	6.2±0.3	3.2±0.1*	2.6±0.1	6.9±0.4	4.7±0.2

rats, and increased in the nuclear fraction. Similarly the total amount of Ca was increased both in the heavy-mitochondrial and in the nuclear fractions from the liver and kidney of deficient animals.

Examination of liver by electron microscopy

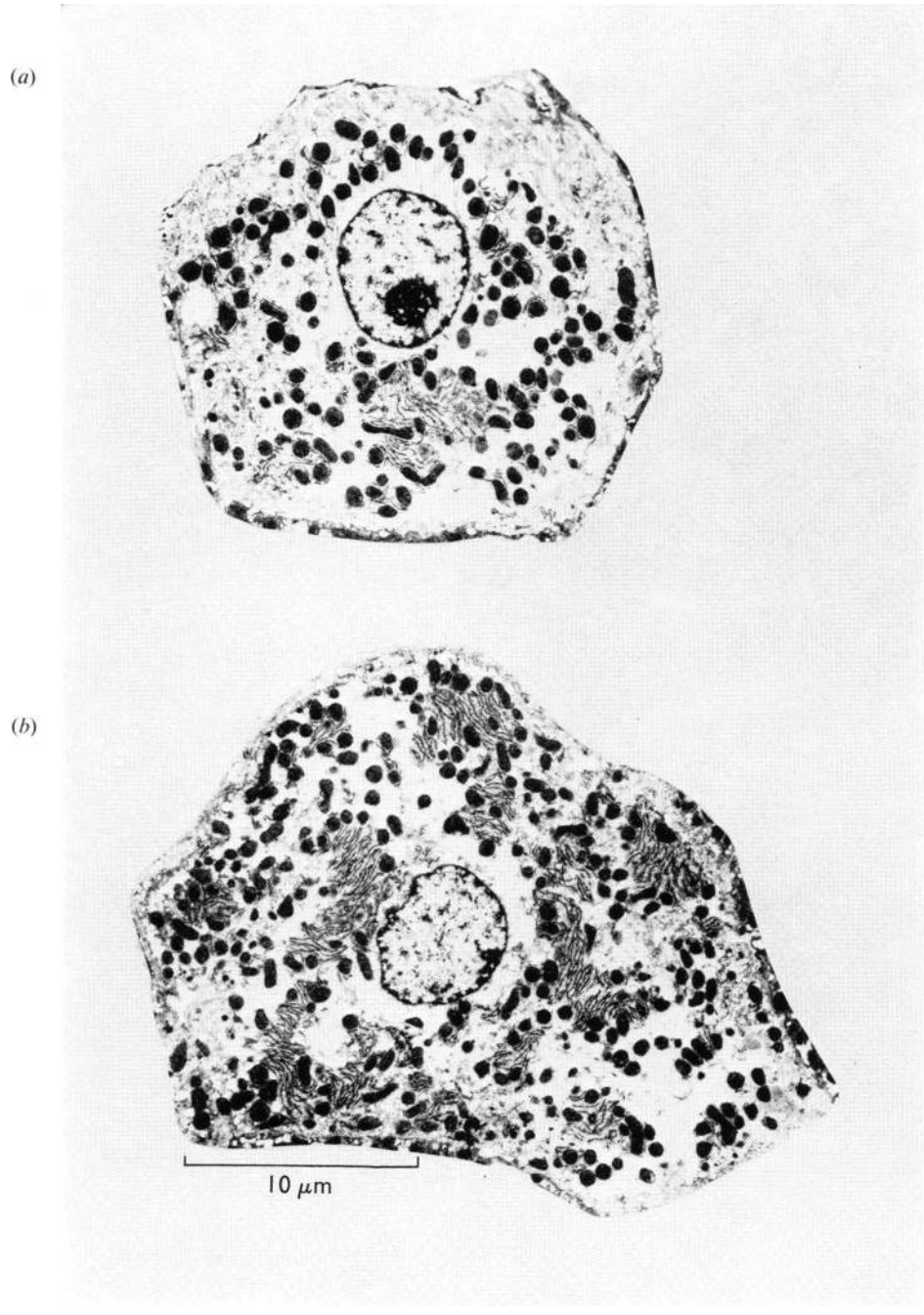
Electron micrographs of liver from the rats in Expt. 2 revealed no evidence of structural lesions, but the average size of the cell and the number of mitochondria within it appeared to be smaller in magnesium-deficient rats than control animals (Plate 1). Preliminary measurements made with a planimeter suggested that differences in cell size were much greater than those in nuclear size, and this was supported by chemical analysis of liver homogenates, which gave values of 13.2±0.3 and 16.2±0.1 mg of nitrogen/mg of DNA ($P<0.001$) for deficient and control rats respectively. Detailed studies with the microdensitometer were therefore concentrated on measurement of mitochondria and cell area.

These measurements indicated that the total areas of a single liver cell and all the mitochondria within the cell were significantly smaller, by amounts of 40 and 47% respectively, in Mg-deficient rats than control animals (Table 6). The number of mitochondria per cell was also decreased in deficient animals to less than one-half of that in controls, and the average area of a mitochondrion was therefore greater in the deficient rats.

Discussion

The results of this investigation indicate that Mg deficiency affects the gross composition of cells in several tissues of the rat and that it has selective effects on particular regions within the cell. The rather limited depletion of cellular Mg, together with some loss of cellular K and the calcification of many tissues (Table 3) is consistent with observations in previous studies of dietary Mg depletion (MacIntyre & Davidsson, 1958; Whang & Welt, 1963; Martindale & Heaton, 1964; Elin *et al.*, 1971), but the decreased proportion of nitrogen in the dry matter and increased water content of all four soft tissues examined indicate that the secondary effects of the deficiency extend beyond mineral metabolism.

This is believed to be the first investigation that has attempted to define the changes during Mg deficiency at the subcellular level, although the distribution of the four major metals between various cellular fractions has been examined in normal animals (Griswold & Pace, 1956; Thiers & Vallee, 1957; Murdoch & Heaton, 1968) and is in general agreement with that found in control rats during the present investigation. Changes in the concentration, rather than the distribution, of a metal



EXPLANATION OF PLATE 1

Electron micrographs of typical liver cells from (*a*) *Mg-deficient and* (*b*) *control rats*

Both cells magnified ×3200. For the purposes of illustration the cells are shown printed on paper of normal contrast.

Table 5. *Distribution of Mg, K, Na, Ca and nitrogen in subcellular fractions of liver and kidney from Mg-deficient and control rats*

Mean values ± S.E.M., $n = 6$ for each group. Values significantly different from control: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

Tissue	Element	Group of rats	Amount (% of that in tissue homogenate)				
			Nuclei	Heavy mitochondria	Light mitochondria	Microsomal fraction	Supernatant
Liver	Mg	Control	16.3±0.3	14.0±0.1	9.2±0.2	45.2±0.6	14.1±0.9
		Mg-deficient	21.7±0.2***	12.8±0.4**	8.9±0.3	45.0±0.5	13.1±0.9
	K	Control	6.7±0.1	2.7±0.1	3.7±0.1	7.9±0.3	79.1±0.6
		Mg-deficient	7.5±0.2**	2.1±0.1***	3.9±0.8	6.5±0.2**	80.2±0.8
	Na	Control	6.8±0.7	2.3±0.1	6.8±0.8	9.1±1.0	74.9±0.5
		Mg-deficient	7.3±0.3	2.3±0.1	6.0±0.9	7.8±1.3	75.1±0.4
	Ca	Control	44.2±0.6	18.1±0.2	16.2±0.9	11.7±0.1	9.4±0.3
		Mg-deficient	46.3±0.2**	20.2±0.2***	13.3±0.9*	12.1±0.4	9.3±0.3
	Nitrogen	Control	24.1±3.7	16.9±1.8	8.2±1.2	18.0±1.1	33.9±0.1
		Mg-deficient	28.7±3.0	9.9±0.6**	9.7±1.9	13.0±0.7**	36.4±0.1***
Kidneys	Mg	Control	13.9±0.5	12.1±0.2	5.2±0.2	22.3±0.4	46.1±1.2
		Mg-deficient	19.9±0.5***	10.5±0.2***	5.1±0.3	21.4±0.4	43.0±0.8
	K	Control	6.6±0.1	5.0±0.1	4.4±0.3	7.0±0.6	76.8±0.9
		Mg-deficient	7.0±0.1***	3.8±0.1***	3.9±0.1	6.2±0.4	78.9±0.7
	Na	Control	7.9±1.3	1.9±0.1	7.8±0.8	6.1±0.4	76.1±0.7
		Mg-deficient	9.0±1.1	2.1±0.1	6.1±0.9	6.8±0.8	74.8±0.9
	Ca	Control	15.8±0.4	12.2±0.3	12.7±1.9	25.2±0.1	32.9±0.4
		Mg-deficient	17.3±0.2**	14.9±0.4***	8.9±0.5	27.2±0.5**	31.7±0.5
	Nitrogen	Control	19.3±1.7	14.6±2.0	8.3±0.9	12.3±1.1	45.0±0.2
		Mg-deficient	18.9±1.9	9.2±0.3*	8.6±0.5	7.4±0.4***	52.2±0.3***

Table 6. *Dimensions of liver cells from Mg-deficient and control rats*

Mean values ± S.E.M. Measurements were made on 50 cells from six individual rats in each group. Values significantly different from control: $*P<0.05$; $***P<0.001$.

Group of rats	Cell area (μm^2)	Total area of mitochondria (μm^2)	No. of mitochondria per cell	Area of single mitochondrion (μm^2)
Control	568 ± 23	168 ± 16	201 ± 6	0.84 ± 0.02
Mg-deficient	338 ± 18***	89 ± 5***	98 ± 2***	0.92 ± 0.03*

are likely to be indicative of a specific effect on its metabolism and the Mg depletion in liver appeared to be the result of selective loss from the mitochondrial fraction (Table 4); however, the rise in Mg concentration observed within the nuclear fraction of both liver and kidney from deficient rats is difficult to understand. The possibility of redistribution of metals occurring during this type of fractionation procedure has been investigated (Murdoch & Heaton, 1968) and found to be improbable, so the information presented in Tables 4 and 5 is likely to be of relevance to the situation *in vivo*.

Histological studies indicate that calcification of the kidney during Mg deficiency is due to deposition of calcium phosphate in and around the cells lining the proximal convoluted tubule (Ko *et al.*, 1962; Heaton & Anderson, 1965). The present evidence for preferential deposition of Ca in the heavy-mitochondrial fraction of liver and kidney during Mg deficiency (Table 4), together with the observation of mitochondrial swelling in the cells of the proximal tubule at an early stage of the deficiency (Hess *et al.*, 1959) suggests that the hypercalcification originates in the mitochondria, which is consistent with the view that this organelle may have a role in the normal calcification process (Lehninger, 1970).

Probably the most noteworthy observation in the present investigation is the change in distribution of material within the liver cell during Mg deficiency, which could be associated with disturbances in the function of particular regions in the cell. A decrease in the size of the whole cell during deficiency is indicated by the results from electron microscopy and the determination of nitrogen/DNA ratios in liver homogenate, but the number of mitochondria within the cell decreased more markedly than the decrease in cell size (Table 6) and was accompanied by a decreased proportion of cellular nitrogen in the heavy-mitochondrial and microsomal fractions of both liver and kidney from deficient rats (Table 5). Moreover, the mean cross-sectional area of the individual mitochondrion was significantly greater in Mg-deficient rats than control animals, which suggests that in addition to decreasing by one-half the number of mitochondria within the liver cell, the deficiency also causes swelling of those that remain. Both the swelling of mitochondria and the disruption of cristae noted by Susin & Herdson (1967) in heart mitochondria from Mg-deficient rats are likely to be associated with an impaired functioning of the organelle, and inhibition of oxidative phosphorylation has been reported in heart mitochondria from Mg-deficient rats and ducks (Vitale *et al.*, 1957; DiGiorgio *et al.*, 1962) although other studies have failed to confirm this (Beechey *et al.*, 1961).

If Mg deficiency does interfere with mitochondrial function the disturbance in energy metabolism could affect many aspects of the cell, including its size and protein content. Alternatively, if the decreased proportion of nitrogen in the microsomal fraction indicates that Mg deficiency selectively inhibits protein biosynthesis, for which there is some evidence in *Aerobacter aerogenes* (Kennell & Kotoulas, 1967; Marchesi & Kennell, 1967), this could have secondary effects on the size of the cell and the number of mitochondria within it. The changes in mineral concentration discussed above suggest that the mitochondria are more likely than the ribosomes to be the location of the primary metabolic disturbance during Mg deficiency, but further work is necessary to confirm this hypothesis.

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