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Magnesium Deficiency in the Adult Rat

By L. MARTINDALE and F. W. HEATON
Medical Research Council Unit for Metabolic Disturbances in Surgery, The General Infirmary, Leeds

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Magnesium deficiency has been extensively studied in young rapidly-growing animals, where it is readily produced by specific dietary restriction. Although the symptoms of deficiency vary slightly between different species it always causes hyperexcitability leading eventually to convulsions (Kruse, Orent & McCollum, 1932; Orent, Kruse & McCollum, 1932; Blaxter, Rook & MacDonald, 1954). The magnesium concentration in the serum falls rapidly and about one-third of the bone magnesium may be lost, but most authors have observed little if any change in the magnesium concentration of soft tissues from either rats (Cunningham, 1936*a*; Watchorn & McCance, 1937; Tufts & Greenberg, 1938) or calves (Blaxter *et al.* 1954). Restitution of magnesium to the diet of magnesium-deficient weanling rats caused a preferential deposition of magnesium in bone (Duckworth, Godden & Warnock, 1940), indicating that the skeletal magnesium acts as a reserve that can be mobilized during deficiency to meet the requirements of other tissues.

Very little information is available about the lability of bone magnesium in the adult animal, but Cunningham (1936*b*) observed normal concentrations in the skeletons of six dairy cows affected by naturally occurring magnesium deficiency. Contrary to the earlier reports, a significant fall in the magnesium concentration of skeletal muscle from young magnesium-deficient rats has recently been found (MacIntyre & Davidsson, 1958; Whang & Welt, 1963). These two considerations stimulated an investigation of the relation between the extracellular, soft-tissue and skeletal magnesium during prolonged deficiency in adult rats, with the object of defining the functional reserve of magnesium in the adult state. Disturbances in calcium and potassium metabolism have been reported during mag-

nesium deficiency (Blaxter *et al.* 1954; MacIntyre & Davidsson, 1958), and the investigation was therefore extended to include a study of the secondary effects of magnesium deficiency on the distribution of sodium, potassium and calcium in the body.

EXPERIMENTAL

Ninety-six adult albino rats of the Sprague–Dawley strain, aged 4–5 months and weighing 190–220 g., were divided into eight groups, each group containing six male and six female animals. The rats were caged individually and received the appropriate diet and distilled water *ad libitum*. Five groups were given a magnesium-deficient diet, and two of the three control groups received the same diet supplemented with magnesium. The diets were prepared as described by MacIntyre & Davidsson (1958) except that casein of low vitamin content (Genatosan Ltd.) was used without acid-washing, and the vitamin solution was mixed with the diet instead of the drinking water. The magnesium content of the diets by direct assay was 0·3 mg./100 g. (deficient) and 40·0 mg./100 g. (control).

The groups of magnesium-deficient rats were killed after 15, 31, 46 and 62 days, the rats in the fifth group being used to replace animals from these four groups that died during the depletion. One group of control animals was killed at the start of the experiment and the others after 31 and 62 days. All rats were killed by exsanguination under ether anaesthesia and the liver, kidneys, heart, thigh muscles, brain, incisor teeth and right femur were removed from each animal.

The effect of convulsions on plasma electrolyte concentration was investigated in a further group of 12 similar rats fed on the magnesium-deficient diet for 5 weeks. Convulsions were stimulated by the sound from a jet of compressed air. Control blood samples were obtained by cardiac puncture under ether anaesthesia 2 days before the convulsions, and further samples were taken at various times after the convulsions.

Sampling of tissues. The tissues from each animal were analysed individually.

Plasma was separated from heparin-treated blood as soon as possible.

Bone was freed from adhering tissue after immersion in hot distilled water for 2 min. The complete femur contained in a silica crucible was dried in an oven at 105° for 16 hr., then ashed in a muffle furnace at 500° for a further 16 hr.; the ash was dissolved in hot 2N-hydrochloric acid. Teeth were dried and ashed in the same way.

For soft tissues, a 10% (w/v) homogenate of each organ was prepared in distilled water in a coaxial all-glass homogenizer. Separate samples of the homogenate were taken for mineral analysis and for the determination of non-collagenous nitrogen. The former was transferred to a silica crucible, dried on a sand bath and dry-ashed by the procedure described for bone.

Analytical methods. Magnesium was determined by atomic-absorption spectrophotometry (Dawson & Heaton, 1961).

Sodium, potassium and calcium were estimated with the Eppendorf flame photometer. The sodium content of all samples and the potassium and calcium in plasma were measured after appropriate dilution with distilled water. Calcium and potassium in bone and soft tissue ashes were measured after dilution with NaH_2PO_4 solution to a final concentration of 0·020M to minimize interference; the blank and standard solutions were treated similarly. For the estimation of calcium in soft tissues the appropriate blank and standard solutions also contained potassium, magnesium, iron and sulphate in amounts corresponding to their occurrence in similar tissues (Long, 1961).

Collagen was removed from tissue homogenates as described by Lilienthal, Zierler, Folk, Buka & Riley (1950), and the non-collagenous nitrogen was determined by the micro-Kjeldahl procedure (Peters & Van Slyke, 1932).

Inorganic phosphorus was estimated by the method of Fiske & Subbarow (1925).

The statistical significance of differences was assessed by the *t* test (Fisher, 1950).

RESULTS

Superficial signs of deficiency. Hyperaemia of the ears was observed in all animals after 12 days on the magnesium-deficient diet, but it faded in the next 6–10 days. About one-third of the rats developed hairless blood-stained skin lesions on the head and neck after 20 days, and these were aggravated by frequent scratching. Diarrhoea occurred in many of these rats. After 24 days on the magnesium-deficient diet all the animals were noticeably hyperexcitable and liable to develop convulsions if stimulated by a sudden noise.

The weight of the control rats increased steadily throughout the experimental period, but the growth of the magnesium-deficient animals was markedly depressed from the start and ceased altogether after 15 days; between 31 and 62 days the mean weight of the deficient animals fell by 8·5% (Fig. 1). The weight of the femurs from the magnesium-deficient rats increased at the same rate as the controls for 46 days, but then growth ceased. Consequently the decline in non-skeletal carcass weight of the deficient animals was even more pronounced than the fall in total body weight (Fig. 1), if the dry weight of a single femur is assumed to have remained an approximately constant proportion (2·5%) of the whole skeletal weight.

Plasma electrolyte concentration. The plasma magnesium concentration fell rapidly from 2·78 to 1·02 mg./100 ml. during the first 15 days, and then more slowly to reach 0·72 mg./100 ml. after 46 days of magnesium deficiency (Table 1). Subsequently it rose significantly ($P < 0·05$) to 1·19 mg./100 ml. in the group killed at 62 days.

The plasma calcium concentration rose progressively from 10·5 to 13·1 mg./100 ml. as the severity of magnesium deficiency increased. This hypercalcaemia was statistically significant ($P < 0·001$) compared with the corresponding control animals after both 31 and 62 days, despite some rise in calcium concentration among the control rats.

No significant change in the plasma sodium or potassium concentrations was observed during magnesium deficiency (Table 1).

Effect of convulsions on plasma electrolyte concentration. A large rise in plasma magnesium and inorganic phosphorus concentrations, together with a smaller rise in calcium concentration, was found within 5 min. of experimentally induced convulsions in magnesium-deficient rats (Table 2). After 4 hr. the concentration of all three ions had returned to approximately the original level. The concentrations of sodium and potassium, together with the specific gravity of the plasma, were unaffected by the convulsions.

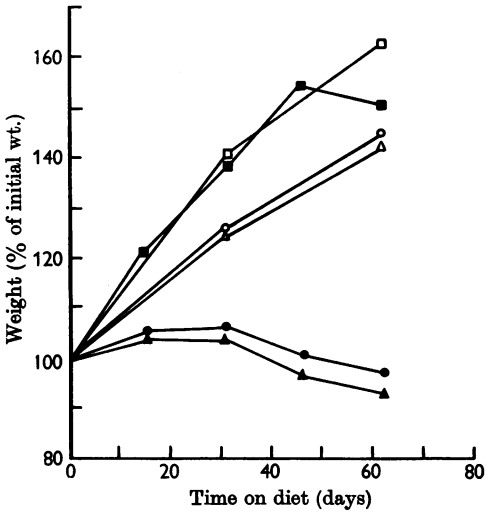

Fig. 1. Changes in the mean total body wt. (○, ●), femur dry wt. (□, ■) and non-skeletal carcass wt. (△, ▲) of control (○, □, △) and magnesium-deficient (●, ■, ▲) rats, expressed as percentages of the initial weights. Experimental details are given in the text. Non-skeletal carcass wt. was calculated from the equation:

non-skeletal wt. (g.) = body wt. (g.) − 40 × single femur dry wt. (g.)

Changes in bone. The magnesium concentration in the femur declined rapidly during the first 15 days of deficiency, and then more slowly to reach 55·5 % of the starting value after 62 days; a small fall in concentration was also observed in the control animals (Table 3). This pattern of change in bone magnesium concentration was similar to that observed in the plasma, and the magnesium concentrations in the plasma and the femur were directly related in both the magnesium-deficient and control rats ($r = +0{\cdot}93$, $P < 0{\cdot}001$, $n = 68$) (Fig. 2).

The considerable femur growth that occurred in the magnesium-deficient rats (Fig. 1) prevents any direct conclusions about the lability of bone magnesium from concentration data. During the first 15 days of deficiency the total magnesium present in the femur fell by 11 %; it then remained essentially unchanged until a further fall of smaller magnitude occurred between 46 and 62 days (Fig. 3). The net loss of magnesium from the femur during the whole period of deficiency amounted to 17 % of the original content.

The changes in other cationic components of bone were less well defined. A significant rise in the concentration of calcium and sodium was observed after 62 days of magnesium deficiency ($P < 0{\cdot}001$ for both cations), and the potassium concentration was raised during the early part of the deficiency ($P < 0{\cdot}001$ at 31 days) but later declined to about the same level as that found in the control animals (Table 3).

The magnesium concentration in the incisor teeth of the deficient rats was also significantly decreased [deficient: 1·31 ± 0·16 % of the ash weight (11 determinations); control: 1·95 ± 0·13 % (12 determinations); $P < 0{\cdot}001$, after 62 days].

Changes in soft tissues. The magnesium concentrations in the liver, heart, thigh muscle, kidneys and brain of the magnesium-deficient rats fell to 91·6, 90·2, 89·0, 87·5 and 87·0 % respectively of the corresponding values from control animals (Table 4). The extracellular magnesium present in the control

Table 1. *Plasma electrolyte concentrations in magnesium-deficient rats*

Experimental details are given in the text. All values are expressed as means ± S.D., with the numbers of determinations in parentheses.

	Concn. of element (mg./100 ml. of plasma)						
	Control rats			Magnesium-deficient rats			
Days on diet ...	0	31	62	15	31	46	62
Magnesium	2·78 ± 0·30 (12)	2·80 ± 0·28 (11)	2·55 ± 0·23 (12)	1·02 ± 0·32 (12)	0·89 ± 0·24 (10)	0·72 ± 0·17 (11)	1·19 ± 0·64 (9)
Calcium	10·53 ± 0·49 (12)	11·11 ± 0·30 (10)	11·52 ± 0·44 (12)	11·81 ± 0·39 (12)	12·53 ± 0·57 (10)	13·10 ± 0·70 (11)	13·11 ± 0·84 (9)
Sodium	324 ± 7 (12)	331 ± 5 (10)	336 ± 7 (12)	331 ± 7 (12)	343 ± 9 (10)	334 ± 7 (11)	340 ± 9 (7)
Potassium	18·7 ± 1·8 (12)	20·2 ± 3·5 (10)	22·3 ± 4·3 (12)	18·9 ± 4·3 (12)	22·0 ± 4·8 (10)	16·5 ± 4·3 (11)	23·2 ± 6·0 (9)

organs, calculated on the assumption that all the sodium was in the extracellular fluid, varied between 2·5 % in thigh muscle and 5 % in kidney of the total magnesium found in the organ. Depletion of extracellular fluid magnesium in the deficient rats would not, therefore, produce large changes in tissue magnesium concentration, and the above values are due chiefly to loss of intracellular cation. The minimum magnesium concentration was usually found after 31 days, and the level then remained constant or rose slightly in some organs. The fall in concentration was significant at the 1 % level for liver, thigh muscle and brain, and at the 5 % level for heart and kidney, after both 31 and 62 days of magnesium deficiency.

Examination of the values for total magnesium content of individual organs shows that the liver behaved differently from the heart and kidneys during the period of magnesium deficiency (Table 5). The total magnesium present in the heart remained unchanged and the kidneys tended to gain magnesium throughout the period of deficiency, although this change was of low statistical significance ($P = 0·1$). The magnesium content of the liver, however, rose by 18 % during the first 15 days and then fell progressively to 88 % of the initial amount at the end of the experiment (Fig. 4). The rate of fall was relatively constant between 15 and 46 days, but it then declined considerably. The magnesium content of the same organs from control rats increased throughout the experimental period.

The total non-collagenous nitrogen present in each organ varied in a manner generally similar to the magnesium content (Table 5). The nitrogen present in the heart and kidneys of the magnesium-deficient rats rose slightly, but the nitrogen content of the liver increased rapidly during the first 15 days of deficiency and then declined progressively (Fig. 4).

The calcium concentration in heart, liver and skeletal muscle was elevated after 62 days ($P < 0·001$, $P < 0·001$ and $P < 0·02$ respectively) but not after 31 days of magnesium deficiency; the calcium concentration in kidney was significantly raised after both 31 and 62 days ($P < 0·05$) (Table 6).

Table 2. *Effect of convulsions on plasma electrolyte concentrations in magnesium-deficient rats*

Control blood samples were obtained from the same rats 48 hr. before the convulsions. Experimental details are given in the text. All values are expressed as means ±S.D., with the numbers of determinations in parentheses.

	Concn. of element (mg./100 ml. of plasma)			
	Control (48 hr. before convulsion)	5 min. after convulsion	4 hr. after convulsion	24 hr. after convulsion
Magnesium	0·62± 0·20 (11)	1·34± 0·43 (9)	0·69± 0·16 (5)	0·69± 0·09 (3)
Calcium	10·0± 0·4 (11)	11·2± 0·5 (9)	10·4± 0·4 (5)	10·3± 0·4 (3)
Inorganic phosphorus	7·0± 0·9 (11)	23·8± 6·6 (9)	6·8± 0·1 (5)	7·3± 1·8 (3)
Sodium	336± 12 (11)	338± 14 (9)	—	—
Potassium	20·6± 3·1 (10)	20·6± 3·1 (6)	—	—
Specific gravity	1·023± 0·001 (10)	1·023± 0·002 (9)	—	—

Table 3. *Femur electrolyte concentrations in magnesium-deficient rats*

Experimental details are given in the text. All values are expressed as means ±S.D., with the numbers of determinations in parentheses.

	Concn. of element (% of ash wt.)						
	Control rats			Magnesium-deficient rats			
Days on diet ...	0	31	62	15	31	46	62
Magnesium	0·669± 0·028 (12)	0·636± 0·023 (12)	0·607± 0·023 (12)	0·481± 0·040 (12)	0·430± 0·042 (11)	0·403± 0·030 (11)	0·371± 0·043 (11)
Calcium	35·8± 0·6 (12)	36·4± 0·5 (12)	36·3± 0·4 (12)	34·0± 1·5 (12)	36·4± 0·6 (11)	37·3± 0·5 (11)	37·9± 0·5 (11)
Sodium	0·684± 0·032 (12)	0·555± 0·013 (12)	0·633± 0·006 (12)	0·571± 0·023 (12)	0·600± 0·026 (11)	0·617± 0·023 (11)	0·698± 0·039 (11)
Potassium	0·224± 0·030 (12)	0·267± 0·022 (12)	0·277± 0·027 (12)	0·521± 0·048 (12)	0·440± 0·036 (11)	0·437± 0·029 (11)	0·314± 0·073 (11)

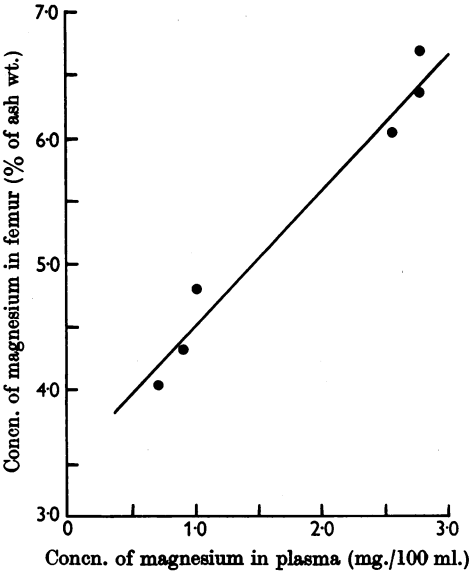

Fig. 2. Relation between the magnesium concentration in the plasma and the femur of magnesium-deficient and control rats. Experimental details are given in the text. The mean values are indicated for each group except for the magnesium-deficient animals killed after 62 days; this group was excluded because of the effect of convulsions on the plasma magnesium concentration.

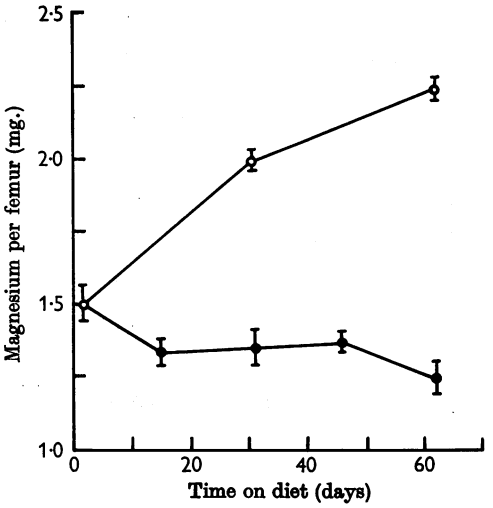

Fig. 3. Changes in the total magnesium content of the right femur from magnesium-deficient (●) and control (○) rats. Experimental details are given in the text. The mean values ±S.E.M. are indicated for each group.

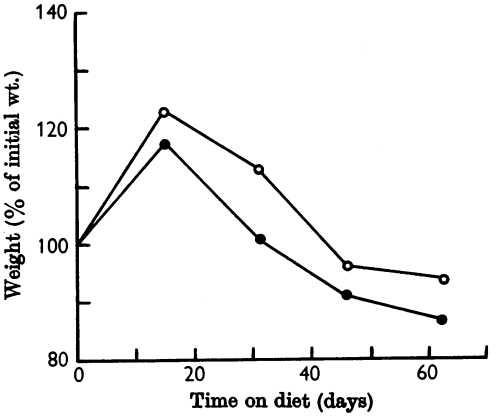

Fig. 4. Changes in the mean total magnesium (●) and non-collagenous nitrogen (○) contents of the livers from magnesium-deficient rats, expressed as percentages of the initial values. Experimental details are given in the text.

Potassium depletion was observed in the liver, heart and thigh muscle after 62 days ($P < 0{\cdot}001$, $P < 0{\cdot}002$ and $P < 0{\cdot}001$ respectively) (Table 6), and a simultaneous rise in sodium concentration occurred in these organs ($P < 0{\cdot}01$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$ respectively).

DISCUSSION

The clinical symptoms of magnesium deficiency in the adult rats were very similar to those observed in weanling animals (Kruse *et al.* 1932; Duckworth *et al.* 1940), but they developed more slowly, probably owing to a proportionately greater growth rate and requirement for magnesium in the younger rats. The magnesium concentration in the plasma fell rapidly at first and then more slowly to reach a minimum after 46 days of deficiency, but it rose considerably in the final group of animals killed after 62 days. The adult rats were relatively resistant to convulsions, which occurred with increasing frequency after 46 days, whereas weanling animals frequently die during the first convulsive episode. Experimentally stimulated convulsions in similar magnesium-deficient rats were followed by a rapid rise in plasma magnesium concentration, thus providing a satisfactory explanation for the raised plasma magnesium concentration found in the severely deficient rats killed after 62 days.

The femur growth, which continued at a normal rate throughout most of the period of magnesium deficiency, together with the small initial rise and subsequent greater fall in the total and non-skeletal carcass weights of the deficient rats (Fig. 1), indicates that changes of magnesium concentration occurring in bone and soft tissues are not necessarily due to the net gain or loss of magnesium, but may simply reflect the growth or atrophy of the

Table 4. *Magnesium concentration in soft tissues of magnesium-deficient rats*

Experimental details are given in the text. Magnesium concentrations are expressed as means ±S.D., with the numbers of determinations in parentheses.

	Concn. of magnesium (mg./g. of non-collagenous nitrogen)						
	Control rats			Magnesium-deficient rats			
Days on diet ...	0	31	62	15	31	46	62
Organ							
Liver	6·7± 0·3 (12)	6·8± 0·3 (12)	6·7± 0·2 (12)	6·5± 0·5 (12)	6·2± 0·4 (10)	6·2± 0·3 (11)	6·2± 0·3 (11)
Kidney	5·5± 1·3 (11)	5·9± 0·4 (12)	6·0± 0·2 (12)	5·2± 0·7 (12)	5·2± 1·0 (11)	5·4± 0·5 (11)	5·8± 0·3 (11)
Heart	7·0± 0·4 (11)	6·5± 0·4 (12)	6·9± 0·3 (12)	6·6± 0·5 (12)	6·1± 0·4 (11)	6·3± 0·6 (11)	6·3± 0·6 (11)
Thigh muscle	8·6± 0·2 (12)	8·3± 0·6 (12)	8·3± 0·2 (12)	8·2± 0·7 (12)	7·4± 0·5 (11)	7·4± 0·4 (11)	7·9± 0·4 (11)
Brain	7·8± 0·6 (12)	7·7± 0·7 (12)	8·5± 0·5 (12)	7·3± 0·6 (12)	7·0± 0·3 (11)	7·4± 0·3 (11)	7·5± 0·3 (11)

Table 5. *Total magnesium and non-collagenous nitrogen in complete organs of magnesium-deficient rats*

All values are expressed as means ±S.D., with the numbers of determinations in parentheses.

		Concn. of constituent (mg./organ)						
		Control rats			Magnesium-deficient rats			
Days on diet		0	31	62	15	31	46	62
Organ	Constituent							
Liver	Magnesium	1·95± 0·37 (12)	2·29± 0·25 (12)	2·35± 0·30 (12)	2·30± 0·24 (12)	2·04± 0·13 (10)	1·77± 0·19 (11)	1·71± 0·22 (11)
	Non-collagenous nitrogen	294± 49 (12)	339± 44 (12)	354± 46 (12)	356± 38 (12)	332± 36 (11)	283± 26 (11)	276± 34 (11)
Kidney per rat	Magnesium	0·279± 0·085 (11)	0·384± 0·058 (11)	0·432± 0·071 (12)	0·294± 0·053 (12)	0·303± 0·065 (11)	0·312± 0·051 (11)	0·323± 0·028 (11)
	Non-collagenous nitrogen	50·4± 8·7 (11)	65·3± 11·2 (12)	71·4± 6·6 (12)	56·6± 7·0 (12)	58·9± 6·3 (11)	57·6± 6·8 (11)	56·0± 5·6 (11)
Heart	Magnesium	0·152± 0·014 (11)	0·166± 0·018 (12)	0·207± 0·022 (12)	0·150± 0·019 (12)	0·147± 0·012 (11)	0·150± 0·013 (11)	0·152± 0·022 (11)
	Non-collagenous nitrogen	21·8± 2·0 (12)	25·6± 1·9 (12)	29·9± 3·8 (12)	22·6± 2·0 (12)	23·9± 1·8 (11)	24·1± 2·2 (11)	24·4± 3·3 (11)

particular organ. Conclusions about the lability of magnesium can therefore only be made from measurements of the changes in total magnesium content of discrete organs during the period of deficiency.

Magnesium depletion was found to be more widespread among soft tissues than observed previously. A significant fall in magnesium concentration was observed not only in skeletal muscle, as reported in younger rats (MacIntyre & Davidsson, 1958; Whang & Welt, 1963), but also in the liver, heart, kidneys and brain. This depletion was relatively small in extent but uniform between different organs, ranging from 8·4% in liver to 13·0% in brain of the values found in control animals.

When the changes in total magnesium content of the liver, kidneys and heart, organs that could be readily removed in entirety, were examined, considerable differences in behaviour during the period of magnesium deficiency were revealed (Table 5). The heart retained its original amount of magnesium, and the kidneys tended to gain magnesium throughout the period of deficiency. The liver, however, showed a rapid gain in both magnesium and non-collagenous nitrogen during the first 15 days, but it then atrophied progressively, the magnesium and nitrogenous contents falling in parallel throughout the remainder of the experimental period. The considerable fall in non-skeletal weight of the animals after 30 days of magnesium deficiency suggests that organs other than the liver must also have atrophied during this time.

The close relation between the magnesium content and the metabolically active mass, as represented by the content of non-collagenous nitrogen, observed during the periods of growth and atrophy in the liver, together with the relatively small and constant fall in magnesium concentration occurring in all the cellular tissues examined, suggests that the growth and maintenance of soft tissues is

Table 6. *Calcium, sodium and potassium concentrations in soft tissues of magnesium-deficient rats*

Experimental details are given in the text. All values are expressed as means ±S.D., with the numbers of determinations in parentheses.

		Concn. of element (mg./g. of non-collagenous nitrogen)					
		Calcium		Sodium		Potassium	
Days on diet		31	62	31	62	31	62
Organ	Group						
Liver	Control rats	1·71± 0·47 (12)	0·94± 0·24 (12)	26·5± 2·7 (12)	24·6± 2·3 (12)	99·3± 5·4 (12)	106·7± 5·3 (12)
	Magnesium-deficient rats	2·02± 0·68 (11)	2·01± 0·80 (11)	28·7± 2·2 (10)	30·9± 6·0 (11)	100·4± 10·1 (11)	93·8± 10·4 (11)
Kidney	Control rats	2·50± 0·57 (12)	2·51± 0·19 (12)	34·1± 2·1 (11)	54·4± 4·1 (12)	101·0± 4·4 (11)	75·8± 4·6 (12)
	Magnesium-deficient rats	16·3± 20·8 (11)	13·2± 20·0 (11)	47·4± 4·9 (11)	55·9± 7·3 (11)	95·9± 9·9 (11)	77·0± 10·7 (11)
Heart	Control rats	3·47± 0·71 (12)	1·44± 0·75 (12)	55·1± 4·2 (12)	42·1± 3·1 (12)	70·6± 7·1 (12)	87·9± 4·8 (12)
	Magnesium-deficient rats	2·22± 0·70 (11)	5·22± 3·31 (11)	53·5± 5·0 (11)	50·3± 7·7 (11)	74·4± 10·0 (11)	71·9± 13·7 (11)
Thigh muscle	Control rats	3·00± 0·57 (12)	1·82± 0·95 (12)	33·3± 2·7 (12)	21·1± 1·8 (12)	98·8± 5·9 (12)	113·3± 3·2 (12)
	Magnesium-deficient rats	3·75± 2·78 (11)	3·85± 2·56 (11)	32·7± 4·9 (11)	33·2± 6·0 (11)	92·4± 13·4 (11)	94·3± 9·5 (11)
Brain	Control rats	6·31± 2·3 (12)	7·30± 2·2 (12)	70·0± 5·3 (12)	58·7± 6·1 (12)	162± 8 (12)	182± 21 (12)
	Magnesium-deficient rats	6·56± 3·7 (11)	7·39± 7·5 (11)	65·4± 8·0 (11)	58·0± 3·2 (11)	182± 9 (10)	177± 9 (11)

dependent on the existence of an approximately normal intracellular concentration of magnesium. The potassium depletion encountered in the liver, heart and skeletal muscle from the rats killed after 62 days on the deficient diet provides further evidence of cellular atrophy during magnesium deficiency. This contrasts with the replacement of potassium by sodium and magnesium found in skeletal muscle from potassium-deficient rats (Hingerty, 1963).

The total amounts of magnesium present in the liver and the femur varied in a generally converse manner throughout the period of deficiency (Figs. 3 and 4). During the first 15 days, the magnesium content of the femur fell by 11 % and the amount in the liver rose by 18 %, but between 15 and 46 days, when no net loss occurred from the femur, the liver atrophied rapidly and its magnesium content fell by 25 %. When a further mobilization of femur magnesium occurred between 46 and 62 days, the rate of loss from the liver was considerably diminished.

As magnesium is lost uniformly from the various parts of the skeleton during deficiency (Duckworth & Godden, 1941), it is clear from the changes observed in the femur that the skeleton provides the principal reserve of magnesium, and that most of the labile fraction can be mobilized without affecting the normal rate of bone growth. The size of this mobilizable reserve, which amounted to 17 % of the total femur magnesium, is relatively smaller in the adult rat than the 30 % loss reported in weanling rats (Duckworth *et al.* 1940), but it is considerably larger than the 4 % of bone magnesium found to exchange with intraperitoneally administered ^{28}Mg in normal adult rats (Breibart, Lee, McCoord & Forbes, 1960). In view of the relatively short duration of experiments with ^{28}Mg, and the good agreement between the 31–40 % of bone magnesium found to be exchangeable with ^{28}Mg in the weanling rat (Breibart *et al.* 1960) and the direct measurements of skeletal mobilization during deficiency in similar rats (Duckworth *et al.* 1940), this implies that the effective skeletal reserve of magnesium is not only proportionately smaller in the adult than in the weanling rat, but also that it can only be mobilized more slowly. When the labile skeletal reserve is exhausted, magnesium is lost from the liver and possibly some other soft tissues, but this loss of intracellular magnesium is only achieved at the expense of tissue atrophy and it does not therefore constitute a true reserve.

The direct relation between the magnesium concentrations in the plasma and the femur of both the control and deficient rats (Fig. 2) is in accordance with observations in younger rats (McAleese & Forbes, 1961) and calves (Smith, 1959*b*). It supports the conclusion that the skeleton provides the principal reserve of magnesium in the body and suggests that an equilibrium exists between the magnesium in the plasma and the bone.

The rise in plasma magnesium concentration occurring immediately after a convulsion is probably due to an increased liberation from bone; the unchanged specific gravity of the plasma excludes any appreciable haemoconcentration, and the

simultaneous rise in magnesium, calcium and phosphate concentrations, without any change in the potassium concentration, indicates the mobilization of skeletal rather than intracellular electrolytes. Orent, Kruse & McCollum (1934) claimed to have demonstrated a simultaneous rise in serum magnesium concentration and fall in bone magnesium concentration after convulsions, but this has been denied (Duckworth *et al.* 1940).

The finding of hypercalcaemia, together with an early and extensive although rather variable calcification of the kidney, and later calcification of the heart, liver and skeletal muscle during magnesium deficiency, confirms previous observations (Tufts & Greenberg, 1938; MacIntyre & Davidsson, 1958). The inverse relation between the plasma concentrations of magnesium and calcium (Table 1) further suggests that the disturbance in calcium metabolism is proportional to the degree of magnesium depletion.

The magnesium concentrations in tissues from the control rats usually remained relatively constant, and, as expected, the total magnesium present in individual organs increased progressively throughout the experimental period. A significant decrease in magnesium concentration ($P < 0.001$) was, however, observed in the femur of rats consuming the control diet throughout the whole experimental period, and this, together with the simultaneous rise in plasma calcium concentration ($P < 0.001$), indicates that the magnesium content of the control diet was suboptimum. The magnesium concentration in this diet (40 mg./100 g.) was appreciably higher than the 15 mg./100 g. needed for optimum growth (Kunkel & Pearson, 1948), and the 36·5 and 35·4 mg./100 g. required, respectively, for the maintenance of normal serum and bone magnesium concentrations (McAleese & Forbes, 1961) in young rats. The calf has been shown to absorb ingested magnesium less efficiently with increasing age (Smith, 1959*a*); if a similar situation occurs in other species this could explain why a higher magnesium content appears to be necessary for the control diet of adult rats.

SUMMARY

1. The effects of dietary magnesium deficiency were studied in adult rats (initial weight 200 g.). Although the animals lost weight, growth of the femur continued at a normal rate until the final stage of deficiency.

2. The magnesium concentrations in the plasma and the femur were directly related; they fell rapidly at first and then more slowly during the later stages of deficiency. The net loss of magnesium from the femur during the whole period of deficiency was 17 % of the original content.

3. A significant fall in magnesium concentration of 8·4–13·0 % occurred in the liver, heart, thigh muscle, kidneys and brain. The total magnesium in the liver increased rapidly at first but later fell continuously; the heart and kidneys retained their initial content of magnesium.

4. Secondary hypercalcaemia, together with calcification of the kidneys and later the heart, liver, skeletal muscle and femur, was observed. Potassium depletion occurred in the heart, liver and skeletal muscle.

5. It is concluded that the skeletal magnesium acts as a reserve, but the fraction mobilizable during magnesium deficiency is smaller in the adult than in the weanling rat. The maintenance and growth of soft tissues appears to require an approximately normal intracellular concentration of magnesium.

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