The Production of Secondary Potassium Depletion, Sodium Retention, Nephrocalcinosis and Hypercalcaemia by Magnesium Deficiency

By I. MacINTYRE and D. DAVIDSSON*
Postgraduate Medical School, Ducane Road, London, W. 12

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Studies of dietary magnesium depletion in the rat have been made by Kruse, Orent & McCollum (1932), Watchorn & McCance (1937) and Tufts & Greenberg (1938). These workers reported an early stage of vasodilatation and hyperexcitability followed by a chronic stage of cachexia. Little or no change was found in the magnesium content of the tissues other than bone, teeth and serum (Watchorn & McCance, 1937), although Tufts & Greenberg (1938) reported minor changes in muscle and brain.

The failure of magnesium-deficient diets to produce changes of magnesium content in the soft tissues contrasts with the marked decreases in muscle potassium produced by potassium deficiency (Heppel, 1939). For this reason it was considered that a fuller investigation of magnesium deficiency was necessary. This has been undertaken by using specific flame-spectrophotometric techniques.

EXPERIMENTAL

Thirty white female rats were placed in two equal groups. The control group (mean initial wt. 101 g.) and the experimental group (mean initial wt. 99 g.) were fed on diets identical in respects other than magnesium content. Rats from each group were killed at intervals by exsanguination under ether anaesthesia.

Ten rats from the control group were killed, at 11, 21, 28, 35, 42, 49, 54, 63 and 70 (two rats) days. None of the control group died. Eight rats from the experimental group were killed at 11, 21, 28, 35, 42, 49, 54 and 62 days. The remaining seven rats of this group died (see Results).

Diet. The diets were made by mixing acid-washed casein (200 g.), arachis oil (80 g.), cod-liver oil (20 g. to which had been added 50 mg. of vitamin E), cane sugar (660 g.) and either salt-mixture C (40 g., control group) or salt-mixture D (36·7 g., experimental group).

Salt-mixture C consisted of NaCl (33 g.), $CaCO_3$ (188 g.), KH_2PO_4 (170 g.), potassium citrate (61 g.), ferric citrate (7·5 g.), 'trace mixture' (1 g.) and $MgCl_2,6H_2O$ (41 g.). In salt mixture D the $MgCl_2$ was omitted.

The 'trace mixture' consisted of KI (13 g.), NaF (10 g.), $MnCl_2,4H_2O$ (2 g.) and Cu_2Cl_2 (0·5 g.).

A stock vitamin solution was prepared and stored at −20°; 250 ml. contained vitamin B_1 (0·03 g.), vitamin B_6 (0·03 g.), biotin (0·006 g.), pantothenol (0·3 g.), *p*-aminobenzoic acid (1·5 g.), inositol (1·5 g.), nicotinic acid (sodium salt; 1·5 g.), choline chloride (4·5 g.), folic acid (0·015 g.), riboflavin (0·12 g.), vitamin B_{12} (0·0003 g.) and vitamin K analogue (Synkavit, Roche Products Ltd., Welwyn Garden City, Herts.; 0·0006 g.).

This solution (16 ml.), made to 2 l. with water, provided sufficient drinking water and water-soluble vitamins for 30 rats for 3 days.

The diets were given *ad lib.* The electrolyte content of the diets (m-equiv./kg.) as found by analysis was: Na, 46; K, 141; Ca, 300; Mg, 36 (control group) and 1·0 (deficient group).

Flame-photometric procedure. Na, K, Ca and Mg were determined in solutions of tissue and plasma ash by flame spectrophotometry at wavelengths of 589, 404, 423 and 285 mμ respectively. Chloride was determined in dry muscle powder and in plasma by measuring the excess of silver flame photometrically at 328 mμ after precipitation of silver chloride (Menis, House & Rains, 1957). Appropriate measures previously described were taken to eliminate interferences (MacIntyre, 1957). The instrument was modified as follows: the earlier burner was replaced by an integral atomizer-burner of the type used in the Beckman flame spectrophotometer (Beckman Instruments Inc., South Pasadena, California, U.S.A.) and described by Burriel-Martí & Ramírez-Muñoz (1957). The range of the instrument was extended to the ultraviolet by an Uvispek monochromator (Hilger and Watts Ltd., London N.W. 1) and quartz-window photomultiplier (type 6256B, E.M.I. Electronics Ltd., Hayes, Middlesex). An oxyhydrogen flame was used except for magnesium. With the authors' instrument satisfactory estimation of magnesium was possible only with oxyacetylene. Under these conditions the interferences were small. With a solution containing 0·5 m-equiv. of Mg/l. and 35 m-equiv. of Na/l. the proportions of the total emission at 285 mμ due to Mg, Na and the flame were 56, 2 and 42% respectively. Phosphate did not interfere.

Sampling techniques

Muscle. Samples were obtained from the thighs, and dried to constant weight at 105° and then ground in a mortar with several changes of a mixture of equal volumes of ethyl ether and light petroleum (b.p. 40–60°). Obvious tendon was removed. The powder was replaced in the oven at 105° for 12 hr. and stored in a desiccator. Duplicate samples (20 mg.) of the dry fat-free muscle powder were weighed into crucibles and ashed at 400°. The ash was dissolved in N-HCl (4 ml./20 mg. of muscle powder) for flame photometry.

Duplicate samples (15 mg.) of the muscle powder were weighed into accurately graduated conical centrifuge tubes for chloride analysis. A volume (1 ml.) of 8N-HNO_3 solution containing 2 m-equiv. of Ag/l. was added and the

* Rockefeller Foundation Fellow. Present address: University of Reykjavik, Iceland.

tubes were heated in a boiling-water bath for 30 min. After cooling, the volumes were made to 4 ml. and the tubes centrifuged. Silver was then determined in the clear supernatant and the chloride content of the original sample calculated.

Collagen was determined in duplicate samples (10 mg.) by the method of Neuman & Logan (1950) but the preliminary extraction with urea solution was omitted.

A further group of 12 female rats (approx. wt. 100 g.) on stock diet were killed by exsanguination (six rats) or by a blow on the head and samples of muscle from the thigh and back obtained for analysis. The carcasses were allowed to remain at room temperature for 18 hr., when second samples of muscle were obtained from each rat. Collagen was not estimated in this group.

Plasma. Heparin was used as anticoagulant. Plasma was separated immediately: 0·02 g. was taken for chloride analysis by the method described for muscle, 0·5–2·0 g. was dried and ashed in platinum crucibles and the ash was taken up in N-HCl (4 ml./g. of plasma) for flame photometry.

Bone. A complete femur was analysed. It was ground, defatted as for muscle and the total dry fat-free weight recorded before ashing with a mixture of equal volumes of HNO_3 and $HClO_4$. After evaporation to dryness the ash was dissolved in N-HCl (100 ml./g. dry fat-free wt.) and analysed for Ca and Mg.

Brain, liver and kidney. These were analysed for Ca, Mg and K by the method used for muscle, after ashing as for bone, except that fat was not extracted from brain.

Statistical analysis. The results were analysed as described by Fisher (1950).

RESULTS

During the first 10 days of the experiment both the control and deficient group failed to grow. Subsequently the controls grew rapidly while the deficient group grew very little and finally lost weight (Fig. 1). After about 12 days the deficient rats developed vasodilatation and oedema of the nose, ears and paws. This lasted for 7–10 days.

Seven of the deficient rats died after 39, 42, 43, 54, 62 (two) and 64 days. In five, samples of bone and muscle were analysed, since they were obtained within a few hours of death. The carcasses of the rats dying at 43 and 54 days were not analysed.

Table 1 shows that the Mg and Ca content of muscle remains constant for 18 hr. after death. Differences between the electrolyte concentrations in Tables 1 (*a*) and 2 (*c*) may be attributable to differences in age of animal (Holliday, Segar, Lukenbill, Valencia & Durell, 1957) and collagen content of sample.

Ion changes in different tissues

Muscle. There was a progressive fall in Mg content of muscle in the deficient group with a highly significant correlation ($r = 0{\cdot}72$, $P < 0{\cdot}01$) between muscle Mg and time on deficient diet. The data were expressed by the equation: muscle Mg (m-equiv./kg. of dry fat-free solids) = 102·6 − 0·353 (days on deficient diet). After 64 days

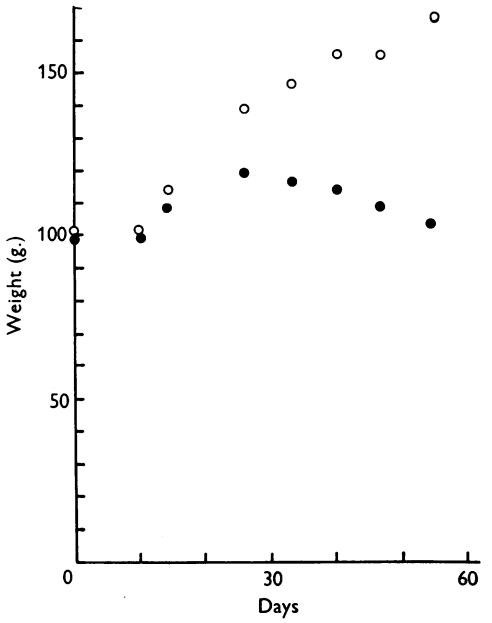

Fig. 1. Growth of control and deficient rats. ●, Deficient; ○, control.

Table 1. *Post-mortem changes in muscle-electrolyte content*

Samples of muscle from thigh and back were obtained from 12 rats, which were then killed. The carcasses were allowed to remain at room temperature for 18 hr., when further samples were obtained from the opposite thigh and opposite side of the back. The figures are m-equiv./kg. of dry fat-free solids. Changes in Ca and Mg are not significant.

	Pre-mortem concn. (mean ± S.E.) (*a*)	Post-mortem concn. (mean ± S.E.) (*b*)	Mean difference ± S.E.M. difference* (*b* − *a*)	*P* (*t* test)
Na	106·1 ± 2·8	128·6 ± 4·6	22·5 ± 5·3	<0·005
K	500·5 ± 6·0	462·6 ± 3·5	−37·9 ± 5·3	<0·001
Ca	10·35 ± 0·19	10·56 ± 0·40	0·21 ± 0·33	>0·5
Mg	103·5 ± 1·3	100·8 ± 2·7	−2·7 ± 1·4	>0·05
Cl	72·5 ± 1·5	122·3 ± 2·7	49·8 ± 3·1	<0·001

* The S.E.M. difference was calculated from the difference in each animal.

the Mg had fallen to 78% of the initial value (Fig. 2).

An unexpected and striking finding was a decline in muscle K (Fig. 3). A highly significant correlation between muscle Mg and K was found ($r=0{\cdot}93$, $P<0{\cdot}01$), those animals which died during the experiment being excluded from this calculation. Results showed a fall in muscle K to 80% of the initial value after 64 days and were described by the equation: muscle K (m-equiv./kg. of dry fat-free solids) = 73 + 3·55 (muscle Mg). After correction for post-mortem changes (Table 1)

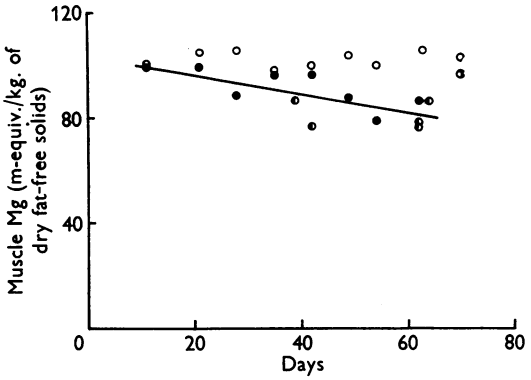

Fig. 2. Magnesium content of muscle. The line is the regression of Mg on time (see text). ●, Deficient rats; ◐, deficient animals which died; ○, control rats.

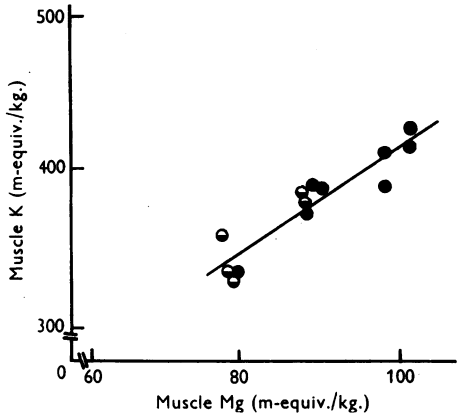

Fig. 3. Relationship of muscle Mg and K. The muscle K contents of the animals which died were corrected for post-mortem fall (Table 1) and are plotted on the graph. ●, Deficient rats; ◒, deficient animals which died.

Table 2. *Electrolyte changes in muscle*

Electrolytes are expressed as m-equiv./kg. of dry fat-free solids. Intracellular Na (Na_i) was calculated as total muscle $Na - \left\{\frac{Na/kg.\ of\ plasma}{Cl/kg.\ of\ plasma} \times 0{\cdot}94 \times Cl/kg.\ of\ muscle\ dry\ fat\text{-}free\ solids\right\}$*. Samples taken from those animals which died are included only for Ca and Mg. For Mg and K in the deficient group the standard errors were calculated by analysis of variance, taking into account the regressions shown in Figs. 2 and 3 respectively.

	(*c*) Control		(*d*) Deficient			
	Days on diet. Mean ± S.E. (no.) range	Mean (± S.E.M.)	Days on diet. Mean ± S.E. (no.) range	Mean (± S.E.M.)	Mean diff. (± S.E.M. diff.)	*P* (*t* test)
Na	44±6 (10) 11–70	91·7±2·9	38±6 (8) 11–62	142·8±7·8	51·1±8·3	<0·001
K	44±6 (10) 11–70	444·3±8·8	38±6 (8) 11–62	396·1±3·7	−48·2±9·6	<0·001
Ca	44±6 (10) 11–70	14·1±2·6	44±5 (13) 11–64	18·7±2·5	4·6±3·6	>0·05
Mg	44±6 (10) 11–70	100·9±1·0	44±5 (13) 11–64	87·1±1·6	−13·8±1·9	<0·001
Cl	44±6 (10) 11–70	59·5±3·6	38±6 (8) 11–62	94·1±3·2	34·6±4·8	<0·001
Na_i	48±6 (9) 21–70	12·7±2·4	42±6 (7) 21–62	11·1±4·9	−1·6±5·5	>0·5
Collagen (% dry fat-free solids)	44±6 (10) 11–70	3·7±0·2	45±5 (13) 11–64	5·7±0·4	2·0±0·4	<0·001

* The factor 0·94 corrects for the difference between plasma water and interstitial-fluid concentrations of Na and Cl due to the Donnan effect. For other assumptions see text.

the results from the animals which died fit the equation (Fig. 3). No such correlation existed in the control group.

The mean electrolyte changes are summarized in Table 2. It will be seen that in the deficient group there were large increases of total Na and Cl, of 51·1 and 34·6 m-equiv./kg. respectively. If the assumption is made that chloride is entirely extracellular (Manery, 1954) the proportion of the total Na which is intracellular can be calculated from a knowledge of the plasma concentration. No difference was detected in 'intracellular' sodium when calculated by this method. This indicates that the extra sodium in the magnesium-deficient animals was entirely extracellular or was accompanied intracellularly by chloride. In neither case could Na have replaced the K lost.

Table 3. *Electrolyte changes in the viscera*

Each value is the mean (no.) ± S.E.M. expressed as m-equiv./kg. of dry fat-free solids except for brain, where the figures are expressed as m-equiv./kg. of dry solids. The mean time on the deficient diet was 38 days. The increase in Ca in the kidneys of the deficient rats is highly significant ($P < 0.01$, t test). The other differences are not significant.

	Kidney		
	Control (*a*)	Deficient (*b*)	Mean diff. (±S.E.M. diff.) (*b* − *a*)
Mg	82·7 (10) ± 1·2	78·8 (8) ± 2·1	−3·9 ± 2·3
K	303·0 (10) ± 3·4	309·3 (8) ± 8·1	6·3 ± 8·7
Ca	12·4 (10) ± 0·5	52·5 (8) ± 13·4	40·1 ± 13·4
	Liver		
	Control (*c*)	Deficient (*d*)	Mean diff. (±S.E.M. diff.) (*d* − *c*)
Mg	81·7 (10) ± 2·5	80·9 (8) ± 1·6	−0·8 ± 3·0
K	352·5 (10) ± 3·0	362·3 (8) ± 5·7	9·8 ± 6·4
Ca	5·2 (9) ± 0·2	5·0 (8) ± 0·2	−0·2 ± 0·3
	Brain		
	Control (*e*)	Deficient (*f*)	Mean diff. (±S.E.M. diff.) (*f* − *e*)
Mg	75·4 (8) ± 1·3	72·4 (7) ± 1·5	−3·0 ± 2·0
K	460·5 (8) ± 4·5	467·3 (7) ± 6·6	6·8 ± 8·1
Ca	19·1 (8) ± 3·1	17·9 (7) ± 1·8	−1·2 ± 3·6

Table 4. *Electrolyte changes in plasma*

Figures are m-equiv./kg. of plasma. The mean time on the deficient diet was 42 days, except for those animals whose plasma was analysed for K, in which it was 32 days.

	Control (*c*) Mean ± S.E. (no.) range	Deficient (*d*) Mean ± S.E. (no.) range	Mean diff. (±S.E.M. diff.) (*d* − *c*)	*P* (*t* test)
Na	140·4 ± 1·5 (9) 135–147	150·4 ± 2·7 (7) 137–157	10·0 ± 3·1	<0·01
K	5·5 ± 1·0 (4) 3·9–8·4	5·2 ± 0·8 (4) 3·5–6·7	−0·3 ± 1·3	>0·5
Ca	5·3 ± 0·1 (9) 4·9–6·0	6·4 ± 0·3 (7) 3·9–8·0	1·1 ± 0·3	<0·01
Mg	1·74 ± 0·09 (9) 1·38–2·16	0·79 ± 0·10 (7) 0·41–1·07	−0·95 ± 0·13	<0·001
Cl	99·2 ± 1·7 (9) 93–107	99·9 ± 3·0 (7) 90–115	0·7 ± 3·3	>0·5

The slight increase in collagen content in the deficient group was too slight to affect interpretation of the other changes.

Kidney, liver and brain. Concentration of Mg did not differ significantly in the two groups in these tissues. Calcium was markedly increased in the kidneys of the deficient animals but normal in the liver and brain (Table 3 and Fig. 5).

Plasma. The deficient group showed reduction in Mg, a slight increase in Na and a marked increase in Ca (Table 4). Fewer analyses were made of K content but no consistent change was seen. Fig. 4 shows that in the deficient group the Mg concentration fell to about 0·4 m-equiv./kg. within 3 weeks and thereafter increased slightly. In Fig. 5 plasma Ca is plotted against renal Ca. Nephrocalcinosis occurred only in the presence of hypercalcaemia.

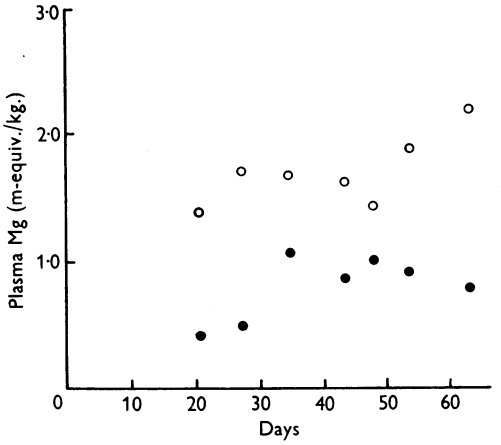

Fig. 4. Plasma Mg concentration. ●, Deficient rats; ○, control rats.

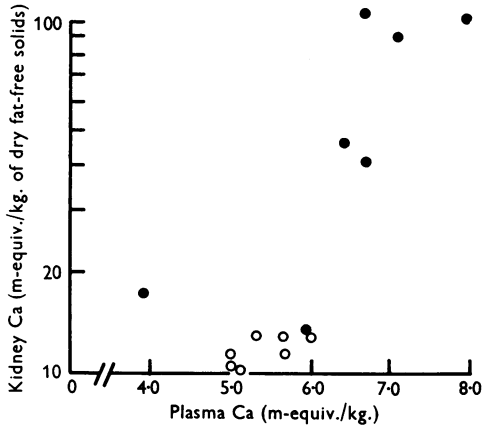

Fig. 5. Relationship of kidney and plasma calcium in ●, deficient rats and ○, control rats.

Bone. Within 3 weeks there was a decline in Mg concentration to about half that of the control group (Fig. 6). Subsequently the concentration declined less rapidly. Fig. 7 shows that during this latter phase the total Mg in the femurs of deficient animals altered very little. The dry fat-free weights of femurs from control and deficient animals were 236 and 234 mg. respectively at 21 days, and 340 and 280 mg. respectively at 64 days.

DISCUSSION

Kruse *et al.* (1932) were the first to describe the characteristic signs of magnesium deficiency in rats. These consist of peripheral vasodilatation and

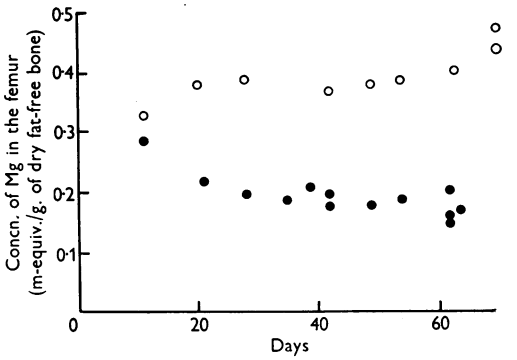

Fig. 6. Magnesium concentration of the femur in ●, deficient rats and ○, control rats.

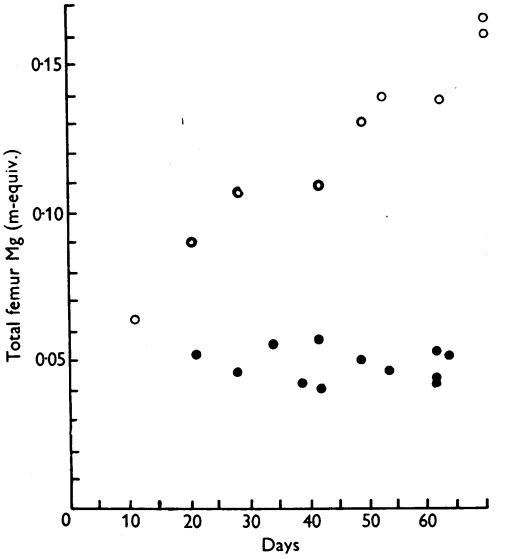

Fig. 7. Total Mg content of the femur. In the deficient animal killed after 11 days total Mg could not be calculated as a portion of the femur was lost. ●, Deficient rats; ○, control rats.

oedema occurring after some days on a deficient diet. They used rats weighing about 50 g. and observed hyperexcitability and convulsions after about 3 weeks. Watchorn & McCance (1937) observed that hyperexcitability did not occur in larger rats. These authors also reported a marked fall in magnesium concentration in bones, teeth and serum. The magnesium content of soft tissues was unaltered except in skin and kidney. In the latter organ a large increase in calcium content occurred. Tufts & Greenberg (1938) found a slight reduction of magnesium in brain and muscle, and an increase of calcium in heart, muscle and kidney. Darrow, Harrison & Taffel (1939) considered that electrolyte analyses were less variable when expressed in terms of dry fat-free solids, a procedure followed in the present experiment and by Cotlove, Holliday, Schwarz & Wallace (1951) but not by previous workers.

In the present experiment little or no significant change in magnesium concentration was detected in brain, liver or kidney, although had the results been expressed in terms of another frame of reference, such as non-collagen nitrogen (Lilienthal, Zierler, Folk, Buka & Riley, 1950), differences might perhaps have been found. In skeletal muscle there was unequivocal evidence of progressive and eventually severe depletion of both magnesium and potassium with a large increase of sodium and chloride. Although potassium depletion is usually associated with an increase in intracellular sodium, this is not invariably found (Holliday & Segar, 1957). Cotlove *et al.* (1951) have described a lesser degree of potassium depletion in rats subjected to milder and less prolonged magnesium deficiency.

It is of interest that depletion of dietary potassium also produces much greater change in potassium content of muscle than of the parenchymatous organs (Darrow & Miller, 1942).

Histological study of the kidneys from the rats in the present experiment suggested that loss of kidney tissue due to nephrocalcinosis was not likely to account for the potassium deficit (Cullen, Davidsson & MacIntyre, 1957, unpublished work) and potassium deficiency still occurred in one of the animals with a normal renal calcium.

Vitale, Nakamura & Hegsted (1957) showed that isolated heart, liver and kidney mitochondria from magnesium-deficient rats were abnormal as judged by the uncoupling of oxidative phosphorylation found under their experimental conditions. Macfarlane & Spencer (1953) showed that rat-liver mitochondria could maintain gradients of sodium and potassium in the presence of an adenine nucleotide and that this ability was linked with their capacity for oxidative phosphorylation and with the prevention of swelling; Bartley & Davies (1954) obtained similar results with sheep kidney. Tapley (1956) has since demonstrated that magnesium protects isolated rat-liver mitochondria from the effects of various agents which cause swelling. Baltscheffsky (1957) found that rat-liver mitochondria swelled within a few minutes of being placed in a magnesium-free reaction medium, and then showed uncoupling of oxidative phosphorylation.

Bartley, Davies & Krebs (1954) point out that mitochondria may be the basic units responsible for active transport in the cell and it is possible that the potassium deficit found in the present experiment was due to an effect of magnesium deficiency on ion transport *in vivo*. A failure from this cause to maintain normal potassium gradients especially, but not necessarily, by the kidney could account for our results.

Fitzgerald & Fourman (1956) attempted to produce dietary magnesium deficiency in man. Two subjects consumed a deficient diet for 20 and 27 days: the serum magnesium did not fall but retention of sodium and chloride occurred. No potassium deficiency was found. It is difficult to draw definite conclusions from this experiment because the diet was deficient in respects other than magnesium concentration, as shown by loss of weight in the control period.

The present findings in muscle bear a resemblance to the effects of primary aldosteronism in man (Conn, 1955). In this condition magnesium and potassium deficiency coexist with sodium retention. Baldwin, Robinson, Zierler & Lilienthal (1952) have noted that magnesium and potassium commonly change together in disease in human muscle, although such a relation was not found in rat muscle when primary potassium deficiency was produced (Cotlove *et al.* 1951).

The changes in bone are as described by Watchorn & McCance (1937). There is some evidence from our results of an absolute decline in the bone magnesium in the early weeks of the deficiency. The production of hypercalcaemia has not previously been recorded in experimental magnesium deficiency although nephrocalcinosis has frequently been detected. In pathological conditions in man nephrocalcinosis is often associated with hypercalcaemia.

Parathyroid hormone is considered to be the main regulator of plasma-calcium level, and although it is possible that hyperparathyroidism was produced in the present experiment no direct evidence of this was obtained.

SUMMARY

1. The effects of dietary-magnesium deficiency have been studied in rats (mean initial weight 100 g.). The animals grew little or lost weight and

after 12 days developed peripheral vasodilatation and oedema which lasted for a further 7–10 days.

2. There was a progressive fall in magnesium content in skeletal muscle to 78 % of the control value after 64 days, described by the equation: muscle magnesium (m-equiv./kg. of dry fat-free solids) = 102·6–0·353 (days on deficient diet). The potassium content of skeletal muscle fell to 80 % of the initial content after 64 days, was closely correlated with muscle magnesium ($r = 0·93$), and was described by the equation: muscle potassium = 73 + 3·55 (muscle magnesium).

3. The mean contents of muscle sodium and chloride increased in the deficient group by 51 and 35 m-equiv./kg. from the control values of 92 and 60 m-equiv./kg. respectively. Assuming all muscle chloride to be extracellular, intracellular sodium in muscle was calculated and found not to differ in the two groups.

4. Magnesium and potassium concentrations were unaltered in brain, liver and kidney.

5. The calcium contents of muscle, brain and liver did not differ significantly in the two groups but there was a mean increase of 40 m-equiv. of calcium/kg. in the kidneys of the deficient group from the control value of 12 m-equiv./kg.

6. Within 3 weeks there was a decline in magnesium concentration in the femur to about half that of the control group.

7. The plasma magnesium concentration fell from the control value of 1·7 to about 0·4 m-equiv./kg. within 3 weeks and thereafter increased slightly. Plasma sodium was slightly increased in the deficient group; the mean calcium concentration in the deficient and control groups was 6·4 and 5·3 m-equiv./kg. respectively.

8. Nephrocalcinosis occurred only when hypercalcaemia was also present.

9. The hypothesis is advanced that the potassium deficiency was due to impaired ion transport secondary to an effect of magnesium deficiency on mitochondrial function.

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