Effect of age on magnesium deficiency in rats

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The development of hypomagnesaemia in calves to the stage at which tetanic convulsions occur has been shown experimentally by Blaxter, Rook & MacDonald (1954) to be a comparatively slow process. In contrast, hypomagnesaemic tetany can develop in the dairy cow and lactating ewe with great rapidity (Rook & Balch, 1958; L'Estrange & Axford, 1963). The increased speed of the development of the condition in the mature animal may be partly due to its inability to mobilize reserves of magnesium from bone as readily as does the young animal (Wilson, 1960).

It was not possible to carry out a controlled experiment to study the lability of Mg reserves in the adult bovine owing to the difficulty in providing a Mg-deficient diet that would support rumination, and to the prohibitive cost of slaughtering animals to obtain tissues for analysis. For these reasons we used rats.

There have already been several reports on the effect of Mg deficiency on the content of Mg in various tissues in the rat. For example, a marked depletion of the Mg content of bone in young rats on a Mg-deficient diet has been described by Duckworth, Godden & Warnock (1940), and MacIntyre & Davidson (1958), under similar conditions, were unable to detect any loss of Mg from soft tissues other than muscle.

In the study to be described here, the effect of maturity on the susceptibility to Mg deficiency and on the availability of Mg reserves has been investigated by feeding young and adult rats of the same strain on a Mg-free diet for 18 days. The development of hypomagnesaemia was followed during the course of the experiment and, after slaughter, the depletion of Mg reserves was determined by analysis of selected tissues. The calcium, sodium and potassium contents of the tissues of adult rats were also studied to see if there were changes associated with Mg deficiency. In addition, the endogenous Mg loss of the adult animals on a Mg-free diet was determined.

EXPERIMENTAL

Animals

Hooded Lister rats which had been maintained on a stock diet (PRM diet as used by the Ministry of Supply, Allington Farm, Porton Down, Wiltshire) were used. The adult rats were males aged 9–12 months and weighed on average 400 g. The young rats were 8 weeks old and of both sexes, the males weighing approximately 190 g and the females 140 g. The animals were housed in cages constructed entirely of Pyrex glass and Perspex to the design of C. H. Gallagher, J. D. Judah & K. R. Rees (1959, personal communication).
Diet

The experimental diets were based on the recommendations of Cuthbertson (1957) and were given ad lib. from Perspex troughs. The composition was (parts) cane sugar 660 (castor), casein (low-vitamin content; Genatosan Ltd) 200, arachis oil 100 and mineral mixture 50 (control diet) or 45 (Mg-deficient diet). The mineral mixture contained (parts): CaCO₃, 75·0; KH₂PO₄, 88·0; NaCl, 24·7; Na₂CO₃, 34·5; FeSO₄·7H₂O, 1·24; MnSO₄·5H₂O, 0·405; ZnSO₄·7H₂O, 0·089; CuCl₂·2H₂O, 0·267; NaF, 0·001; KI, 0·0013; KBr, 0·0004; H₂MoO₄, 0·0008, and only in the control diet MgSO₄·7H₂O, 25·7.

All constituents of the mineral mixture were AR except manganese sulphate and zinc sulphate which were Specpure quality (Johnson Matthey & Co. Ltd). No special purification was undertaken.

Fat-soluble vitamins were dissolved in the arachis oil to give (g/kg diet): vitamin A acetate 0·0012, DL-α-tocopheryl acetate 0·28, ergocalciferol 0·0005. Water-soluble vitamins were given in the drinking water. The quantities (g) dissolved in 4·5 l. distilled water were: thiamine 1·2, riboflavin 0·4, calcium pantothenate 4·0, cyanocobalamin 0·002, nicotinic acid 4·0, folic acid 0·04, biotin 0·008, pyridoxine 0·32, inositol 9·0, choline chloride 40·0, p-aminobenzoic acid 3·0, dicalcium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid (Synkavit; Roche Products Ltd) 0·08, together with chlortetracycline hydrochloride 0·16. To prevent bacterial growth this solution was kept at −20°, and before use it was thawed and diluted (15%, v/v) with distilled water. Sufficient drinking mixture for at least 1 week was prepared.

Experimental design

Adult rats. Forty-eight adult rats were divided randomly into two groups of twenty-four. One group was given the Mg-deficient diet and the other group the control diet. All animals were housed six to a cage except for twelve on the deficient diet. The latter were housed in pairs and, after allowing 2 days for the excretion of Mg originating from the stock diet, their excreta were collected on tinned trays for five successive periods of 3 days and analysed for Mg. To reduce the quantity of material for ashing, spilt diet uncontaminated with excreta was removed daily from the trays.

Six animals from each group of twenty-four were bled when the Mg-deficient diet was introduced and again after 4, 8, 11, 14 and 18 days. The rats were bled in rotation so that no animal was bled for the second time until the fifth bleeding. Blood was removed by cardiac puncture through a 0·8 x 15 mm needle into a heparinized syringe by the method described by Burhoe (1940). From each rat 3 ml of whole blood were taken to allow analysis of individual samples. All rats were weighed weekly.

On the 18th day after the introduction of the deficient diet each rat was given an intraperitoneal injection of 22Mg containing 1 mg stable Mg as carrier. Four rats, two from each dietary group, were killed by decapitation at intervals up to 22 h after dosing. The following tissues were removed immediately after death: brain, heart, liver, both kidneys, muscle (inner thigh), left femur and the left side of the mandible.
Apart from the livers, which were analysed individually, tissues from a pair of rats with a common killing time and dietary history were pooled for analysis.

The results for the uptake of \(^{28}\text{Mg}\) are described in another paper (Field & Smith, 1964).

**Young rats.** Six (two ♂ and four ♀) and twelve (four ♂ and eight ♀) rats were given the control and Mg-deficient diets respectively. Three of the control and six of the deficient animals were bled on the 1st and 15th day of the experiment and the remaining animals on the 8th day. After 18 days on the diets the rats were bled and killed by decapitation. The kidneys, left femur and the left side of the mandible were removed for analysis.

**Analytical procedure**

The heparinized whole blood was centrifuged immediately and the plasma was separated and stored at \(-20^\circ\) until analysed for Ca and Mg.

Soft tissues were dried at 60° to constant weight and ashed in silica beakers for the measurement of Mg, Ca, Na and K.

Bones were dried to constant weight at 100°, ashed in Pyrex glass beakers and their Mg content was determined.

Combined total faeces and urine from each pair of rats for each 3-day period were ashed and analysed for Mg.

All samples except plasma were ashed with 5 ml conc. HNO\(_3\) (AR) until evolution of brown fumes ceased. This process was repeated until little carbon remained, and the ashing was completed with a mixture of equal volumes of HClO\(_4\) (60%, AR) and conc. HNO\(_3\). Water (20 ml) was added and the mixture boiled and filtered while hot into a volumetric flask.

Ca was determined by the method of Henley & Saunders (1958). After removal of Ca as oxalate, Mg was precipitated as MgNH\(_4\)PO\(_4\) from the supernatant solution. The precipitate was centrifuged, washed once with 33% (v/v) NH\(_4\)OH and the Mg determined in either of two ways. For plasma and bone the MgNH\(_4\)PO\(_4\) was dissolved in two drops of 50% (v/v) conc. HNO\(_3\) and transferred to a cuvette with about 30 ml water. After addition of 0·5 ml 2% (w/v) KCN, 3 ml 3N-NH\(_2\)OH and 0·2 ml Eriochrome black T in 2-methoxy ethanol (1 mg/ml), the mixture was titrated stepwise with standard EDTA solution (1 m-equiv./l.) in a Unicam SP 350 spectrophotometer at 620 m\(\mu\), the solution being mixed by a stream of air. The end-point was determined from the plot of extinction against volume of titrant.

For the remaining samples the MgNH\(_4\)PO\(_4\) was dissolved in HNO\(_3\) as above and determined in the same manner as Ca in the method of Henley & Saunders (1958).

Na and K were determined with the EEL (Evans Electroselenium Ltd) flame photometer after suitable dilution of the ashed solution.

**RESULTS**

**Clinical signs.** The first sign of Mg deficiency was reddening of the ear which took slightly longer to develop in the adult (11–14 days) than in the young (8–11 days) rats. Hyperirritability developed in both groups 2–3 days after the ear reddening.
Convulsions were seen in four of the young but in none of the adult rats. No attempt was made to induce convulsions.

The weight curves of the groups of rats are shown in Fig. 1. The weights of the Mg-deficient adult rats remained almost constant whereas those of the controls increased throughout the experiment. The young rats gained weight on both diets, but much more rapidly on the control than on the deficient diet.

Effect of carrier dose of Mg. The carrier dose of 1 mg Mg caused no apparent increase in the Mg concentration in the tissues taken from rats killed at any time after dosing. The time of killing was therefore ignored and the results were pooled for statistical analysis.

Plasma Mg. The mean values found for the concentration of Mg in plasma of the groups of rats are shown in Fig. 2. The introduction of the Mg-deficient diet resulted in a marked reduction in the plasma Mg concentration, the mean values for adult and young rats falling to 0.49 and 0.55 mg/100 ml respectively in 8 days and thereafter remaining constant till the end of the period of observation. No significant change was observed in the mean values for animals on the control diet.

Endogenous Mg excretion. The mean values with their standard errors for the total daily excretion of Mg by the adult rats on the Mg-free diet for the five successive col-
Collection periods were (mg/rat day) $1.17 \pm 0.044$, $0.90 \pm 0.025$, $0.82 \pm 0.020$, $0.70 \pm 0.067$ and $0.81 \pm 0.032$ respectively. They fell significantly from the first to the second period ($P < 0.01$) and from the second to the third period ($P < 0.05$), and thereafter remained constant.

**Bones.** The values obtained for the Mg content of the femur and mandible are given in Table 2. There was a significant ($P < 0.005$) decrease in the concentration of Mg in both the mandible and femur of both the young and adult rats given the Mg-deficient diet. The decrease was significantly ($P < 0.01$) greater in the young than in the adult rats. It will be noted that there was a significantly ($P < 0.01$) greater concentration of Mg in the femur of the young control rats than in that of the adult controls.

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![Fig. 2. Effect of the control and Mg-free diets given ad lib. on plasma Mg concentrations in adult and young rats. Mean values for: •—•, adult rats on the Mg-free diet; •—•, control adult rats; A—A, young rats on the Mg-free diet; Δ—Δ, control young rats.](image)

**Table 1. Mean values with their standard errors for concentration (g/100 g dry matter) of magnesium in bones of young and adult Mg-deficient and control rats and the degree of depletion in the deficient rats**

<table>
<thead>
<tr>
<th>Bone</th>
<th>Young rats</th>
<th>Adult rats</th>
<th>Depletion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient</td>
<td>Control</td>
<td>Depletion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(%I</td>
</tr>
<tr>
<td>Femur</td>
<td>$0.38 \pm 0.014$ (11)</td>
<td>$0.52 \pm 0.014$ (11)</td>
<td>28.2</td>
</tr>
<tr>
<td>Mandible</td>
<td>$0.49 \pm 0.0074$ (11)</td>
<td>$0.73 \pm 0.044$ (11)</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Figures in parentheses are the numbers of observations.
Table 2. Mean values with their standard errors for the concentration (g/100 g dry matter) of calcium, magnesium, sodium and potassium in various tissues of groups of rats after 18 days on the Mg-deficient and control diets

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Deficient diet</th>
<th>Control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>Mg</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.024 ± 0.0011</td>
<td>0.003 ± 0.0014</td>
</tr>
<tr>
<td>Liver</td>
<td>0.0097 ± 0.0015</td>
<td>0.005 ± 0.0016</td>
</tr>
<tr>
<td>Kidney: adult</td>
<td>0.044 ± 0.0063</td>
<td>0.057 ± 0.0019</td>
</tr>
<tr>
<td>young ♂</td>
<td>0.78 ± 0.029</td>
<td>(4)</td>
</tr>
<tr>
<td>young ♀</td>
<td>2.22 ± 0.048</td>
<td>(7)</td>
</tr>
<tr>
<td>Heart</td>
<td>0.0060 ± 0.0005</td>
<td>(11)</td>
</tr>
<tr>
<td>Brain</td>
<td>0.062 ± 0.0093</td>
<td>(12)</td>
</tr>
</tbody>
</table>

Figures in parentheses are the numbers of observations.
Soft tissues. The mean concentrations of Mg, Ca, Na and K in brain, heart, liver, kidney and muscle are shown in Table 2. There were no significant differences between the control and deficient groups of adult rats in the Mg content of any of the tissues examined. In the young rats, however, there was a significant ($P < 0.01$) reduction in the Mg content of the kidneys from rats fed on the Mg-free diet. In addition, the Mg content of the kidneys from young rats on both diets was significantly ($P < 0.01$) higher than that of the kidneys from adult animals treated similarly.

The only detectable effect of Mg deficiency on the Ca content of soft tissues was in the kidney. Some of the kidneys from adult male rats showed increased Ca content whereas others remained normal. The range for Ca content was 0.024–0.101 g/100 g dry matter for the deficient animals and 0.020–0.038 g/100 g dry matter for the controls. On the other hand, kidneys from the young male rats showed a consistent and much larger increase in Ca content, from a mean of 0.025 to one of 0.078 g/100 g dry matter in response to the Mg-deficient diet. The Ca content of the kidneys of the young female rats was very variable; it ranged from 0.20 to 2.5 % in the control group and from 0.73 to 4.1 % in the deficient group.

Significant ($P < 0.05$) differences existed between the two groups of adult rats in the Na content of the heart and muscle, the deficient animals having a higher concentration in both tissues. The K content of the brains of the deficient animals was significantly ($P < 0.05$) higher than that of the control group.

DISCUSSION

The changes in plasma Mg resulting from feeding rats on a Mg-free diet were found to be independent of the age of the rat. Despite the fact that the minimal values reached after a week on the diet were similar in both young and old rats the clinical signs of deficiency were more severe in the young animals. Young rats, for example, showed redness of the ears and increased excitability 2–3 days earlier than the adults, and four of them were seen in convulsions. It will be noted that after a week on the Mg-free diet the concentration of Mg in the plasma was no longer a measure of the severity of Mg depletion.

Of the tissues examined, only bone showed a reduction in its Mg concentration, thus confirming the views of previous workers that the reserves of Mg in the body are confined mainly to the skeleton. The reduction in concentration of Mg in bones over a period of 3 weeks on the Mg-free diet was much less in the adult than in the young rat. It was, in the femur for example, 28.2 % in the young and 9.4 % in the adult rat.

In order to calculate the total amount of Mg liberated from bone, the weight of the skeleton must also be known. Since more than 99 % of the Ca in the body is confined to the skeleton its weight can be calculated from the concentrations of Ca in the body and in bone. From the extensive data for concentration of Ca in the body of rats of various weights given by Sherman & MacLeod (1925) the amounts of Ca in the groups of rats at the beginning and end of the experimental period respectively were calculated to be (g) 1.3 and 1.7 for the young females; 1.7 and 2.2 for the young males; 4.2 and 4.2 for the adult male rats. The concentration of Ca in the skeleton was taken to be
22%, this value being the mean concentration of Ca in femur and mandible of both young and adult rats (Smith & Field, unpublished). On the basis of the above values, and the concentration of Mg in the skeleton (taken as the mean for mandible and femur, Table 1) the young females lost 4 mg, the young males 6 mg and the adult male rats 13 mg Mg. Thus it would appear that the lower availability of skeleton Mg in the adult rat is more than compensated for by the greater weight of skeleton. It was assumed that the rate of skeletal growth of the young rats on the Mg-free diet was normal. If no skeletal growth took place during this period the amount of Mg liberated by the skeleton would be of the same order in both young and adult rats. To obtain the total reserves, the small amounts of Mg lost from the extracellular fluids must be taken into account. If it is assumed that the extracellular fluid accounts for 20% of the body-weight (Darrow & Hellerstein, 1958), the amounts of Mg lost from this source were about 0.6 and 1.2 mg for the young and adult rats respectively.

The requirement for Mg of the adult rat is essentially that for maintenance, i.e. for making good the endogenous loss; any increase in weight is due to deposition of fat which contains negligible quantities of Mg. The total endogenous loss over the 18 days cannot be determined exactly because of the carry-over of Mg from the previous stock diet. An approximation, however, may be calculated from the mean daily endogenous loss found for the last 9 days on the diet, when it was relatively constant at 0.78 mg/rat daily. This calculation gives a value of 14 mg, which is of the same order as that calculated above for the loss of Mg from the skeleton. No attempt was made to estimate the Mg requirements of the young rat, which are determined not only by maintenance but also by growth.

From the 2nd to the 9th day the daily endogenous Mg excretion fell from 1.17 to 0.82 mg/rat, and thereafter remained constant. This observation indicates that the maintenance requirement of the mature rat is reduced when there is a shortage of dietary Mg, but this effect is strictly limited. The plasma Mg, the source of endogenous Mg loss, showed the same pattern but the reduction was relatively greater (from 1.25 to 0.5 mg/100 ml). The value obtained for endogenous loss in the adult rat is equivalent to 2 mg/kg body-weight daily, which is of the same order as losses obtained for cows (3.5 mg/kg (Blaxter & McGill, 1956), 1.5 mg/kg (Simesen, Lunaas, Rogers & Liwick, 1962)), for calves (0.5-2.2 mg/kg (Smith, 1959)) and for sheep (2.0-3.6 mg/kg (Field, McCallum & Butler, 1958), 2.1-5.1 mg/kg (Care, 1960)).

A striking feature of Mg deficiency in the young rat is a disturbance in Ca metabolism as evidenced by an increased Ca content of certain soft tissues and of kidney in particular (Watchorn & McCance, 1937; Tufts & Greenberg, 1937-8; MacIntyre & Davidsson, 1958). The last workers reported a fourfold increase in Ca content of kidneys from female rats. On the other hand, Watchorn & McCance (1937) using a mixture of males and females found a much greater but more variable increase, the Ca content of individual kidneys approaching 3% on a dry-matter basis.

Our results for kidney present a complicated picture. The Ca contents of the kidneys of the young female rats were similar to those reported by Watchorn & McCance (1937) except that, although the Mg-deficient diet appeared to increase the Ca content, the difference was not significant owing to the large variation within both
dietary groups. The values for the young male rats, on the other hand, were much less variable within each group and there was a thirtyfold difference between deficient and control animals. The effect of Mg deficiency was much less in adult than in young male rats, only one kidney showing a fourfold increase over the mean value for the controls.

No change in the Mg and K content of muscle has been found in the work described here, in contrast to the results of MacIntyre & Davidsson (1958) who found a secondary depletion of K in muscle, which showed a highly significant positive correlation with the reduction of Mg. However, these workers have given no values for the K content of muscle after 18 days on the diet but, since at this time they were unable to show any loss of Mg, it follows that there was also no change in K concentration. Thus the differences between their results and ours can be attributed to the shorter duration of our experiment.

There was an increase in the muscle Na of the deficient adult rats in agreement with the results of MacIntyre & Davidsson (1958) for young rats. In addition we found an increase in the Na content of hearts from the deficient animals. In contrast to MacIntyre & Davidsson (1958), we found an increased K content in the brain of Mg-deficient rats.

**SUMMARY**

1. Young and adult rats were given for 18 days a semi-synthetic diet free of magnesium, or the same diet supplemented with Mg. Plasma Mg levels were determined at intervals during the experimental period, after which the animals were killed and Mg was determined in plasma and selected tissues. The content of sodium, potassium and calcium in soft tissues and the endogenous losses of Mg were measured in adult rats.

2. Vasodilation of the ears and hyperirritability took 2–3 days longer to appear in adult than in young rats on the Mg-free diet. Convulsions were seen only in young rats.

3. The concentration of Mg in plasma and the endogenous loss of Mg of adult rats given the Mg-free diet fell during the first 8 days and thereafter remained constant at 0.5 mg/100 ml and 0.7 mg/rat day respectively. Changes in plasma Mg of young rats were similar.

4. After 18 days the mean concentration of Mg in the femur and mandible of the deficient group had decreased by 9.4% and 13.8% respectively in adults and 28.2% and 33.3% respectively in young rats.

5. No changes in the Mg mean concentrations of the brain, heart, liver, kidneys and muscle were detected in the deficient adult group. The kidneys of the young rats showed a reduction in Mg content in the deficient group.

6. The concentrations of Na in heart and muscle and of K in brain were significantly higher in the deficient adult group than in the controls.

7. An increased concentration of Ca in the kidney due to Mg deficiency occurred in young males but not in young females or in adult males.

8. The differences in the effects of the Mg-free diet on mature and young rats are discussed.
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