CLXXII. SUBACUTE MAGNESIUM DEFICIENCY IN RATS

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THE first attempt to deprive an animal of magnesium appears to have been made by Osborne & Mendel [1918] during the course of a general study of the inorganic elements in the nutrition of the rat. Their "magnesium-free" diet still contained over 100 parts Mg per million and no untoward effects were apparent. Leroy [1926] tested a diet containing only 10 parts Mg per million on a small group of mice and found that growth ceased after 9–13 days and that 3 out of 5 of the mice died in 26–35 days. Acute Mg deficiency, produced in both rats and dogs by a diet containing only 1·8 parts Mg per million, has been studied by McCollum and co-workers and described in a series of papers [McCollum & Orent, 1931; Kruse, Orent & McCollum, 1932, 1, 2; 1933; Orent *et al.* 1932; 1934; Kruse, Schmidt & McCollum, 1933; Klein *et al.* 1935]. The main features of the experiments on rats were the production of acute hyperaemia of the skin and loss of hair, convulsions and death in many cases as early as the 11th day. Lavollay [1932] obtained similar effects with rats on a diet containing 30 parts Mg per million and Suguira & Benedict [1935] have recently confirmed these results. Cramer [1932] and Brookfield [1933] have also briefly described the results of a partial deficiency of Mg in rats. Cramer's diet contained over 60 parts Mg per million. None of the acute effects noted by Kruse *et al.* [1932, 1] were observed, but after 6 weeks on the diet the animals suffered from albuminuria, nephritis and calcareous deposits in the kidney. Brookfield's rats did not show any skin lesions but died in or after convulsions in 4–6 weeks. *Post mortem* there were pathological changes in the liver and kidneys, but no calcification in the latter. The liver changes were regarded as an important effect of the deficiency. Greenberg & Tufts [1934–35] state that with a diet containing 10–20 parts Mg per million the time of onset of the convulsions depended upon the level of vitamin B_2 in the diet and that with ample supplies of this vitamin there were no trophic changes such as loss of hair, emaciation and oedema of the feet.

The experiments described below have been in progress for the last 3½ years and form part of a study of subacute Mg deficiency at various levels of intake of the mineral.

Experimental

Most of the present work was carried out with standard piebald black and white rats bred in the Cambridge Biochemical Laboratory. The Glaxo strain of the Wistar albino rat was used in one group of experiments. Approximately 150 animals of both sexes have been studied.

The diet was as follows:

Casein (Glaxo ash-free) ...	23
Cane sugar	17
Rice starch	40
Lard	15
Salt mixture	5
	100

The salt mixture for the controls consisted of the slightly modified McCollum and Davis mixture commonly used in the Cambridge laboratory [Watchorn, 1932]. A similar salt mixture, omitting only the $MgSO_4$, was used for the deficient group. Vitamin B complex was supplied by an extract of dried yeast in doses corresponding to 0·5 or 0·75 g. of dried yeast per rat per day, according to age of the rat. The yeast was extracted with 65% alcohol, the latter evaporated *in vacuo* and the residue made up with distilled water so that 1 ml. was equivalent to 1 g. dried yeast. 2–3 drops of cod-liver oil were also given daily to each rat. Beyond omitting the $MgSO_4$ from the salt mixture no attempt was made to purify the diet further. It contained approximately 40 parts Mg per million. Food was allowed *ad libitum* and distilled water was supplied in glass bulbs. The cages were $12 \times 9 \times 10$ in. with false screen bottoms; generally two rats were housed in each. The initial weights of the rats varied between 55 and 83 g. in the case of the main, long-term experiments. Younger and older animals were also used in some of the short-term special experiments. The long-term experiments lasted for 3 months. It was evident that a continuation beyond this would have resulted in serious loss of appetite and weight, effects which in themselves might have affected some of the final results.

General results

Abnormal signs were first noticed about the 10th day; in the case of the piebalds diarrhoea then started. The faeces were black and tarry and gave intense reactions for blood. The animals were dull, dirty and sickly, tended to lose appetite and weight and sometimes seemed at the point of death. After a few days the skin became hyperaemic. There was loss of fur along the flanks, under the chin and at the back of the neck. With the onset of the hyperaemia there was rapid, even dramatic, improvement in the general condition. Diarrhoea ceased, appetite became excellent, and in spite of the skin condition the rats became lively and playful, often in the course of 2–3 hr. The loss of fur was followed by a scaly condition with some bleeding and the skin became hardened and thickened in patches, though the rest of the skin appeared abnormally thin and translucent, a condition also noticed by Lavollay [1932]. The changes in the skin were evidently irritating and the bleeding and thickening may merely have been the result of scratching. The skin lesions soon began to clear up and had generally disappeared in 1 week. Neither they nor the diarrhoea reappeared and the rats remained in good health and general condition until near the end of the 3-month period. In the albino rats the diarrhoea was slight or absent but the skin lesions were more intense than in the piebalds. Convulsions never occurred in the piebald groups and only those animals started on the diet at 35–40 g. weight showed any increased excitability. These younger rats however had hyperpnoea with fine tremors over the whole body. Recovery was slow when they were placed on a normal diet. Three of the albino rats, each weighing approximately 65 g., had convulsions of the type described by Kruse *et al.* [1932, 1]. These rats, unlike the younger ones with hyperpnoea and tremors, quickly recovered when restored to a normal diet for a few days and the acute symptoms did not recur when Mg was again withheld. Until near the end of the experimental period all the rats ate well; growth was, however, somewhat subnormal, though not nearly to such an extent as in the rats of Kruse *et al.* [1932, 1] (Figs. 1 and 2). At about the 10th week appetite generally began to decrease slightly, the fur became harsh and discoloured and the rats were dull and listless. Very slight oedema of the paws and genitals occurred in a few instances only, and there was no albuminuria

apart from haematuria. The latter was sometimes severe; it occurred at irregular intervals from the 6th week onwards and each attack lasted several days.

The signs of Mg deficiency varied a little from one litter to another but considering the results as a whole the intensity of the early effects did not appear to be influenced by sex although each separate litter generally showed sex differences within itself. Later in the experiment the general appearance of the males in all groups was definitely worse than that of the females and this was corroborated by the post-mortem findings.

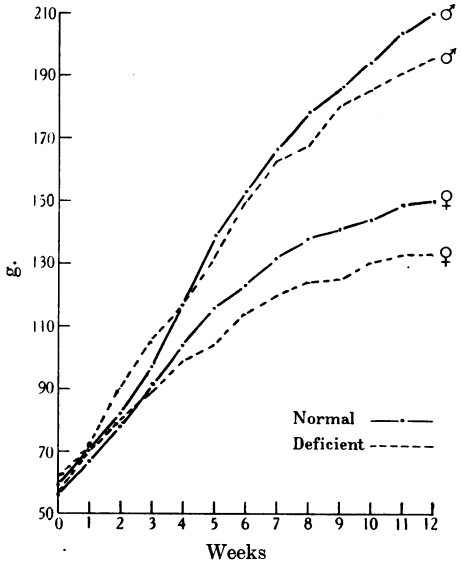

Fig. 1. Piebald rats.

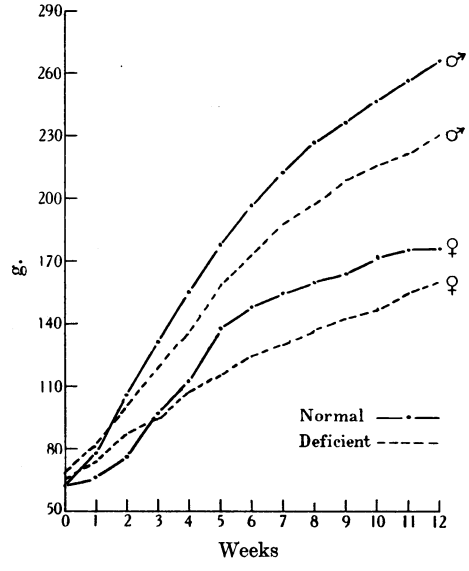

Fig. 2. Albino rats.

There was one striking feature of the deficiency visible during the life of the animal, which has not previously been described. After a few days on the diet a very pronounced and characteristic smell became evident. This was not faecal in character but was aromatic, pungent but not unpleasant. It appeared to be given off by the whole surface of the body. Later a sticky, reddish exudate appeared on tails, paws and ears. In the piebalds this was generally slight and might have been overlooked, but in the albinos it was often of extraordinary intensity so that the whole of the cage became thickly coated with it and had to be cleaned frequently. On the outer edges of the ears it had the appearance of dried blood and in fact was for a time accepted as such until confirmatory tests were applied. This exudate was associated with the odour described above, although the odour was noticed before the exudate became evident. The exudate contains approximately 4 % P and seems to consist partly of a keratin-like protein and partly of a lipoid. The phenomenon has been observed in other conditions (e.g. vitamin B_2 deficiency) but only to a comparatively mild degree.

Post-mortem appearances

The deficient animals showed the following abnormalities after 90 days on the diet: (1) The subcutaneous (as distinct from the fat in the skin) and abdominal fat was diminished, particularly when the skin lesions were at their worst. The

fat in the skin itself is discussed later. (2) Small haemorrhages were found in some of the males, usually in the bladder but occasionally also in the wall of the stomach. (3) The appearance of the kidneys was variable. Some were practically normal, other grossly abnormal. The worst were pale yellowish brown or mottled, soft and flabby in consistency. In two males only kidney stones were present. (4) Stones were found in the bladder of three males. (5) The long bones were very brittle, especially near or at the epiphyses, so much so that it was difficult to remove a knee-joint intact. The bones appeared to be of normal length but were purplish red in colour. The naked-eye changes are difficult to describe but were so obvious that a third person could pick out the abnormal bones from a mixed collection. (6) The teeth of the piebald rats appeared normal or nearly so. The incisors were sometimes paler than normal and had a somewhat translucent appearance. The incisors of the albinos were brittle and rather loose in their sockets and occasionally they were chalk-white. The molars were outwardly normal.

Histological findings

(1) *Kidney.* After 3 months a few calcareous deposits scattered throughout the cortex and medulla were the only lesions visible in many of the kidneys. In the most severely damaged calcareous deposition was more extensive; calcareous casts were present in the straight and collecting tubules, producing obliteration of the epithelium lining and sometimes cystic dilatation of the tubules above the level of the cast. Mild inflammatory reaction was occasionally observed in the neighbourhood of the deposits and in a few kidneys haemolysed blood was present in the capsular space of the glomeruli and convoluted tubules. On the whole it can be said that there were no lesions indicative of any type of nephritis: glomerular changes were completely absent.

(2) *Liver.* Normal after 3 months on the diet.

(3) *Skin.* The skin of the trunk and ears was examined after 12–15 days on the diet when the dermatitis was acute. There were focal areas of necrosis and ulceration of the epithelium with an acute inflammatory reaction in the subjacent dermis.

(4) *Tail.* When examined at the height of the skin lesions the tail showed excessive keratinization and desquamation of the outer layers.

(5) *Bones.* The long bones and rib junctions were examined after 3 months and in spite of the brittleness and naked-eye changes no definite histological lesions were observed. It is possible that they should have been examined earlier when the growth rate was more rapid.

(6) *Teeth.* The histological changes were extensive and important and will be described in full elsewhere. Briefly the most striking feature of the incisors was the irregular striated appearance of the dentine which was no longer stained evenly with haematoxylin but showed rings which had taken the eosin and haematoxylin alternately. The odontoblasts showed all stages of degeneration and in some parts they had disappeared. The capillaries were enlarged. The molars were also affected and the pulp cavities contained very numerous pulp stones which were entirely absent from the normal controls. The first slight changes were apparent after 2 weeks on the diet.

Chemical investigation

Methods and procedure

(1) *Serum.* The Ca and Mg were determined as previously described [Watchorn, 1933] and the phosphatase by Jenner & Kay's method [1932].

(2) *Tissues.* The various tissues were removed as rapidly as possible, wiped from adhering blood with filter-paper and weighed in small tared bottles. Pooled tissue from 3 rats was used for each analysis. Skeletal muscle was taken from the hind legs. A wide band of skin encircling the whole body was removed from between fore- and hindlimbs. Subcutaneous fat was dissected away and the hair clipped off very close to the skin. All samples were dried to constant weight and fat determined on the skins by the usual Soxhlet extraction. Ca, Mg, Na and K were determined as described by McCance and Shipp [1933].

(3) *Bones and teeth.* For each separate bone analysis one whole forelimb and one whole hindlimb were taken, without the digits. The bones were dried to constant weight, then thoroughly extracted in a Soxhlet apparatus, first with alcohol and then with ether. The fat-free bones were again dried to constant weight and finally ashed in platinum crucibles. The Ca in the ash was determined by McCrudden's method [1910] and P by Fiske & Subbarow's [1925]. The Mg was determined in the filtrate from the Ca precipitate according to Franklin [1932–33]. Phosphatase was determined in the bones after they had been dried in a desiccator and on the fresh kidneys according to the technique very kindly given by Prof. Kay in a personal communication. In essential details this was similar to that previously described by him [1926].

Chemical results

(1) *Blood.* The serum Mg of the deficient rats was invariably reduced, the average being less than half the normal (Table I). The difference between the

Table I. *Serum Mg*

	No. of rats	mg./100 ml. Range	Mean	S.D.
Normal ♂ and ♀	9	3·60–4·81	4·26±0·16	0·49
Deficient ♂ and ♀	21	1·35–2·90	2·01±0·11	0·49
Deficient ♂	12	1·35–2·38	1·80±0·12	0·41
Deficient ♀	9	1·50–2·90	2·18±0·19	0·56

averages for male and female animals was not statistically significant but the higher serum Mg level of the females is interesting in view of their better condition compared with that of the males. 13 of the rats to which the figures in Table I refer were killed after 3 months on the diet, but equally low values were found in those killed after 2 and 4 weeks only. In fact the lowest value of all (1·35 mg./100 ml.) was found in a rat killed after only 12 days on the deficient diet. The normal serum Mg fell within the range previously established for the colony [Watchorn, 1933]. The serum Ca of both normal and deficient animals was also within the normal range.

(2) *Tissues. Water* (Table II). The kidneys, liver and skins of the deficient animals showed a tendency towards increased percentages of water but only the figures for the kidneys are statistically significant ($t=3·36$, $n=8$, P lies on the 0·01 level). The average water content of the normal skins (55·8 %) was rather higher than that (51·2 %) found by Fawns & Jung [1933]; the water content of the normal hearts exactly corresponded with the figures given by MacKay & Bergman [1932].

Fat. This was determined only in the skins. It averaged 8·3 % in both the normal and deficient males. In the females the normal value was higher (16·8 %) and the value for the deficient skins was 12·4 %.

Table II. *Water of tissues*

		Brain	Gut	Heart	Kidney	Liver	Lung	Muscle	Skin
Normal	No. of analyses	3	1	4	5	3	5	6	8
	Mean %	77·4	67·4	76·2	74·3	67·6	78·2	72·8	55·8
	S.D.	0·06	—	0·41	1·32	1·55	0·38	1·09	4·78
Deficient	No. of analyses	3	3	4	5	3	5	6	8
	Mean %	76·1	65·3	76·1	76·9	70·7	78·1	72·6	57·9
	S.D.	0·30	2·60	0·56	1·09	3·51	0·97	1·37	4·60

Magnesium (Table III). It is at once obvious that individual tissues of the deficient rats contained little, if any, less Mg than those of the normals. With the exception of the liver, however, all the average values of the deficient rat tissues were slightly lower than those of the controls. The decrease, calculated on a

Table III. *Mg of tissues*

	Fresh weight						Dry weight					
	Normal			Deficient			Normal			Deficient		
Tissue	No. of analyses	Mean mg. per 100 g.	S.D.	No. of analyses	Mean mg. per 100 g.	S.D.	No. of analyses	Mean mg. per 100 g.	S.D.	No. of analyses	Mean mg. per 100 g.	S.D.
Brain	3	19·6	1·76	3	18·8	1·60	3	86·1	6·80	3	83·0	6·95
Gut	4	23·7	1·88	4	22·0	3·90	1	68·4	—	1	52·8	—
Heart	5	24·2	5·38	5	22·4	4·65	4	93·5	15·00	4	90·3	21·45
Kidney	6	23·0	1·49	6	19·6	1·51	5	87·4	6·50	5	83·8	6·84
Liver	3	21·6	0·58	3	24·9	2·29	3	66·7	4·69	3	86·2	19·72
Lung	5	18·5	3·77	5	18·2	5·80	5	84·8	17·43	5	82·9	23·35
Muscle	8	27·9	3·16	8	26·8	5·70	6	102·2	8·52	6	91·2	20·50
Skin	7	9·3	0·49	7	7·8	1·01	5	29·6	5·22	5	24·9	4·79

fresh weight basis, is significant for both skin and kidney. (For skin $t=3{\cdot}54$ and for kidney 3·92; in both cases P is less than 0·01.) The average values for each tissue were obtained from pairs of figures, each pair of which represents the analysis of pooled organs of a normal and a deficient group, the two groups being made up of rats of the same sex and equally distributed litter-mates. Taking all the tissues together there were 41 such pairs, and of these 31 showed a decrease of Mg in the deficient group. It seems reasonable to believe on this evidence that there was a tendency towards a real, but slight, decrease of Mg in brain, gut, heart, skeletal muscle, lung and skin of the rats which had received the deficient diet. The increase in liver Mg of the deficient rats was found in all three cases, but does not represent a statistically significant difference from the normal.

The normal skin values corresponded to those given by Loewy & Cronheim [1932] but were appreciably lower than those of Fawns & Jung [1933].

Calcium (Table IV). The Ca figures tended to be irregular in several organs, particularly in the brain. The striking feature was the enormous concentration of Ca in the kidneys of the deficient animals, amounting in one group to over 3 % of the dry weight of tissue. This calcification has been referred to in the histological section and will be discussed later. Skeletal muscle Ca was increased in the deficient group and may be significant since for the fresh weight series, $t=2{\cdot}60$ and P is approximately 0·02. For the dry weight series the significance is higher, $t=3{\cdot}57$ and P is less than 0·01.

The normal values for skin agreed with those found by Fawns & Jung [1933] and the normal muscle Ca, if one high value is omitted, agrees with the figures given by Burns [1933].

Table IV. *Ca of tissues*

	Fresh weight						Dry weight					
	Normal			Deficient			Normal			Deficient		
Tissue	No. of analyses	Mean mg. per 100 g.	S.D.	No. of analyses	Mean mg. per 100 g.	S.D.	No. of analyses	Mean mg. per 100 g.	S.D.	No. of analyses	Mean mg per 100 g.	S.D.
Brain	3	55·9	46·70	3	45·4	55·87	3	247·8	207·10	3	201·5	248·40
Gut	4	14·2	2·67	4	24·0	19·43	1	36·9	—	3	73·8	61·39
Heart	5	10·7	4·46	5	10·2	4·04	4	38·1	13·06	4	37·8	15·28
Kidney	5	21·8	11·75	7	328·2	177·50	4	92·2	54·68	7	1446·0	821·40
Liver	5	7·3	2·09	5	7·7	2·74	3	19·0	3·85	3	20·6	7·85
Lung	5	19·0	3·79	5	24·1	16·59	5	87·2	16·76	5	108·9	72·11
Muscle	7	13·0	7·28	7	25·4	10·33	5	45·3	28·48	5	113·0	31·44
Skin	6	11·4	2·68	6	11·8	3·45	3	35·2	5·41	3	34·4	1·36

Sodium and potassium (Table V). The variations between the K values of normal and deficient animals were small and occurred in both directions. The skin showed the greatest change, the average normal value being 0·162 % and the average deficient values 0·127 % (fresh weight). These differences however

Table V. *Na and K of tissues*

		Fresh weight						Dry weight					
		Normal			Deficient			Normal			Deficient		
Tissue		No. of analyses	Mean mg. per 100 g.	S.D.	No. of analyses	Mean mg. per 100 g.	S.D.	No. of analyses	Mean mg. per 100 g.	S.D.	No. of analyses	Mean mg. per 100 g.	S.D.
Brain	Na	2	110	—	2	112	—	2	486	—	2	492	—
	K	3	440	87	3	433	126	3	1950	382	3	1966	480
Gut	Na	4	102	29	4	112	37	1	442	—	3	360	149
	K	4	391	41	4	433	189	1	1350	—	3	1339	820
Heart	Na	4	108	15	4	111	25	4	454	56	4	476	123
	K	5	359	33	5	398	16	4	1532	145	4	1717	12
Kidney	Na	3	144	33	3	126	23	—	—	—	—	—	—
	K	6	362	109	6	324	70	3	1700	206	3	1667	206
Liver	Na	4	76	3	4	70	7	2	231	—	2	224	—
	K	5	421	119	5	425	110	3	1491	449	3	1634	695
Lung	Na	4	161	21	4	157	13	4	732	95	4	726	37
	K	5	293	34	5	334	77	5	1345	156	5	1522	332
Muscle	Na	4	63	11	4	61	11	2	258	—	2	246	—
	K	6	434	65	6	450	77	4	1630	310	4	1778	371
Skin	Na	5	131	16	5	130	9	5	419	110	5	429	64
	K	5	162	9	5	127	49	5	520	138	5	420	220

are not statistically significant, owing to the comparatively great range of variation shown in the deficient group. Most of the tissue figures, in fact, showed considerable variation. The Na values for the two groups were in good agreement with each other. It can probably be concluded that the metabolisms of both Na and K were unaffected by Mg deficiency.

(3) *Bones* (Table VI). The bones of the deficient rats contained more water and less Mg than the controls. Water was unfortunately only determined in the albinos but the increase (2·4 %) in the deficient group was significant, $t = 3{\cdot}22$ and P less than 0·01. The Mg in the deficient rat bones was rather less than two-thirds the normal value. On the other hand the ash, Ca and P showed practically no changes.

(4) *Teeth* (Table VII). A more complete analysis of teeth at different levels of Mg intake is being made but the results of the examination of a few lower incisors are here included. As the numbers are few and the individual variations were very small, the standard deviation has been omitted from the table.

Table VI. *Bones*

	Water %	Ash: % of fresh wt.	Ash: % of fat-free dry wt.	Ca: % of fresh wt.	Ca: % of fat-free dry wt.	Ca: % of ash	Mg: % of fresh wt.	Mg: % of fat-free dry wt.	Mg: % of ash	P: % of fresh wt.	P: % of fat-free dry wt.	P: % of ash
						Normal						
Mean	29·5	39·4	59·0	14·9	22·4	38·0	0·38	0·58	0·98	7·4	11·0	18·6
No. of analyses	10	10	18	10	18	18	10	18	18	10	18	18
S.D.	2·02	2·13	1·32	0·91	0·73	1·00	0·04	0·05	0·08	0·58	0·94	0·53
						Deficient						
Mean	31·9	37·7	60·0	14·8	23·1	38·5	0·20	0·37	0·61	6·9	10·8	18·1
No. of analyse	11	8	18	11	21	18	11	21	18	11	21	18
S.D.	1·59	1·09	2·06	0·65	0·94	1·76	0·04	0·09	0·15	0·45	0·49	0·46

Table VII. *Teeth*

	Water %	Ash: % of fresh wt.	Ash: % of fat-free dry wt.	Ca: % of fresh wt.	Ca: % of fat-free dry wt.	Ca: % of ash	Mg: % of fresh wt.	Mg: % of fat-free dry wt.	Mg: % of ash	P: % of fresh wt.	P: % of fat-free dry wt.	P: % of ash
						Normal						
Mean	15·3	61·4	75·2	22·2	27·6	36·5	1·00	1·24	1·65	12·2	15·1	20·1
No. of analyses	3	3	5	3	5	5	3	5	5	3	5	5
						Deficient						
Mean	18·6	60·1	76·2	22·6	29·1	37·2	0·55	0·65	0·86	11·9	13·9	18·4
No. of analyses	3	3	2	4	4	5	2	4	5	2	2	5

In the deficient teeth the water content was increased while the Mg was only half of the normal value. There was a small but definite decrease of P. Murray [1936] for normal rats of a similar age obtained higher Mg (2·36 %) and lower Ca (34·82 %) values. The diet used by her contained a higher level of Mg than did ours. There seems to be a reciprocal relationship between these two minerals in teeth, for if the figures are converted into "total" Ca (1 g. Mg ≡ 1·648 g. Ca) the value obtained by Murray was 38·72 % and by us 39·2 %. The respective Ca/P "corrected" ratios are 1·97 and 1·95. The corresponding "corrected" ratio for the deficient teeth would be 2·10, the increase being caused by the diminished P, since the "total" Ca for the deficient teeth was slightly lower (38·6 %) than for the controls.

(5) *Phosphatase.* Cramer [1933–34] suggested that the large amount of Ca in the kidneys of Mg-deficient rats might inhibit the phosphatase activity. A few determinations were therefore made, in each case on a grossly abnormal kidney. Determinations were also made on a few samples of plasma and bone. No inhibition of phosphatase activity was found in the pathological kidneys and the enzymic activity of the bones and plasma from the deficient animals was normal.

Discussion

The earliest visible effects of a partial deprivation of Mg are changes in the peripheral blood vessels. This results in hyperaemia of the skin with loss of hair, and in haemorrhages in the alimentary tract. There is in some animals hyper-excitability and, rarely, convulsions. The severity of these early manifestations is largely related to the age of the rat and, except in very young animals, they

rapidly disappear. In connexion with the skin lesions, it may be mentioned that low serum Mg values have been found in a variety of human skin diseases (unpublished results). Permanent changes brought about by the dietary shortage of Mg consist of deficiency of this mineral in the blood, bones and teeth, and in calcification of the kidneys, though it is not yet certain that the latter is not due to the combined effect of the Mg deficiency and some other dietary maladjustment. Some of the animals, for instance, show signs of vitamin B_2 deficiency, although the normal controls receiving the same amount of vitamin do not, suggesting that possibly the requirements of vitamin B_2 are higher in certain mineral deficiencies or that vitamin B_2 functions less efficiently in the absence of a sufficiency of Mg. In considering the present results in the light of other published work it is evident that the kidney changes described by Cramer [1932] have not been entirely confirmed, although the kidneys of our rats also show calcification and other abnormalities. Cramer reported that extensive damage was obvious after only 6 weeks from the start of the experimental diet, whereas in the present series of experiments all the kidneys examined at the end of the 6th week were still normal. Moreover, in Cramer's animals the glomeruli were the first parts to be affected whilst in our animals even the worst of the kidneys showed, at most, only slight glomerular changes. We have never found true albuminuria except in association with haematuria. In any case the histological picture strongly suggests that the changes are the result of a preliminary precipitation of Ca in the tubules. The kidney of the rat seems to be liable to this type of abnormality and Cramer found calcification in half of his control animals. Calcification of the kidneys is not a lesion specific to any particular deficiency or dietary maladjustment as it has been found in many conditions. For example McCarrison produced kidney stones in addition to bladder stones with diets deficient in vitamin A and containing excess of Ca [1927, and several other papers in the same *Journal*]. Polak [1934] produced kidney stones with high Ca intake, Watchorn [1932] with high Mg intake, and Gough *et al.* [1933] with high P levels and excess vitamin D. Since many of the control rats in Cramer's series also showed calcification of the kidney it is possible that some other deficiency existed along with that of Mg. The supply of vitamin B, for example, appears to have been inadequate. The basal diet used by Brookfield [1933] seems to have been still more open to criticism, for his control animals not only did not grow normally but actually lost weight or remained stationary. It is quite certain that here Mg was not the only deficiency. MacKay & Oliver [1935] criticized the lesions found by Cramer on the ground that his basal diet contained sufficient P to cause the renal damage. In the various diets used by MacKay and Oliver the lowest concentration of P which led to kidney damage appears to have been 0·74 % of the whole diet, while in Cramer's case it was approximately 0·61 % and in the diet used by McCollum and co-workers it was roughly 0·4 %. (These calculations have been made by us from the available data; none of these authors expressly states the P contents of the whole diets.) In our own diet the P was approximately 0·5 %. Our control animals all had healthy kidneys but it is of course possible that when the Mg content of the diet is severely reduced ill effects may follow from the disturbed Mg/P ratio quite apart from the effects of the absence of Mg *per se*. This point needs investigation. It may provide the explanation of the discrepant results which have been obtained in regard to the renal effects of Mg deficiency.

One of the most unexpected features of the present experiments has been the fact that the organs of the deficient animals contained normal, or only slightly subnormal, amounts of Mg. The very slight decrease in Mg in many of the organs

may be due entirely to the low level of Mg in the blood which they contained when they were taken for analysis. The liver, on the other hand, in spite of its large content of blood, actually showed an increase of Mg. The idea that certain of the soft tissues might part with Mg in order to maintain the more essential ones was not substantiated. One can only conclude from the present results that the Mg in all these tissues is not only essential to their continued existence but must be maintained at a constant level. Definite deficiencies of Mg were found only in the blood, bones and teeth. This is in agreement with the experience of Cunningham [1936–37] who found that dietary variations of Mg affected the bones and blood only (he did not examine the teeth). It is remarkable that the rats were able to settle down to an uneventful life with a blood level of Mg so far below normal. After the first 3 weeks there did not appear to be a state of even "latent tetany". The deficient rats were quite undisturbed by loud or sudden noises or by being handled, in fact sometimes they were rather dull and lethargic.

It is difficult to compare the present bone results with those reported by Orent *et al.* [1934] because their rats were younger when placed on the diet and when finally examined, and the diet itself was more deficient in Mg. Their rats were 55 days old when sacrificed and had been on the experimental diet 30 days only, whereas our rats were approximately 130 days old and had been on the diet 90 days. Orent *et al.* considered that the increases in ash and Ca occurred in the deficient bones during the first few days on the diet and their curves showed that the difference between these bones and the normals tended to decrease with age. Taking these facts into account it is perhaps not surprising that our animals as a whole showed no changes in bone Ca.

We have seen that the chemical changes in the teeth are greater than those in the bones. The reduction in Mg is greater, the increase in water is relatively greater and the P is decreased. With regard to the increased percentage of water, it may be mentioned that Clarke & Smith [1935] found a still larger increase in the teeth of rats deprived of salts in general. It is interesting to note that in spite of the fact that the Mg of the deficient teeth is only half the normal value, the level is still actually higher than in the normal bones (fresh weight). A higher concentration of Mg seems to be necessary for normal tooth structure than for normal bone. Not only are the permanently growing incisors affected by a deficiency but also the molars, which in rats are of limited growth. Klein *et al.* [1935] have briefly described the changes induced by their more extreme diet. They consider that the specific effect of the lack of Mg is on the "paradontium" and the adjacent alveolar bone, for the molars of their rats were embedded in a mass of tissue, possibly of inflammatory origin. This did not occur in our case and quite definitely the first changes were capillary dilatation and odontoblastic degeneration. The suggestion of Klein *et al.* that the dentinal striations are the result of the convulsive attacks and remissions is quite untenable since all rats on the diet show the same dental abnormalities irrespective of the occurrence of convulsions. Further comment on the teeth is postponed until the publication of greater details.

The question arises whether there is any common factor in the various changes brought about by Mg deficiency. One fundamental characteristic appears to be the capillary injury and dilatation. Evidences of this are the melaena, the haematuria, the hyperaemia of the skin, the increased water contents of the kidneys, skin and teeth and the capillary dilatation in the latter. We are perhaps dealing with a condition resulting primarily from disturbed capillary permeabilities but the syndrome produced appears to be characteristic only of a shortage of this particular mineral.

SUMMARY

1. Rats weighing 50–85 g. maintained on a diet containing approximately 40 parts of Mg per million (4 mg./100 g.) developed diarrhoea and melaena beginning about the 10th day. This was followed by hyperaemia of the skin, loss of hair and in some cases hyperpnoea and nervousness. These symptoms passed off in 7–10 days and thereafter the rats appeared normal until near the end of the experimental period (12 weeks). They then lost appetite and began to look out of condition.

2. *Post mortem* the kidneys were found to be calcified, the bones brittle and the teeth sometimes white or translucent.

3. The blood Mg was reduced to approximately half its normal value within 14 days on the diet. It remained at this level, no further reduction occurring.

4. The soft tissues contained only very slightly less Mg than normal. The difference was not statistically significant.

5. The water contents of the bones, teeth and kidneys were significantly increased.

6. The Mg content of the bones was reduced to approximately two-thirds and that of the teeth to one-half of the normal level. In addition the P of the teeth was reduced and extensive histological changes were present.

7. The phosphatase of blood, bones and kidneys was normal.

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