THE EFFECTS OF EXCESSIVE INTAKE OF MAGNESIUM BY THE RAT; ESPECIALLY CONCERNING THE FACTORS RELATING TO THE PRODUCTION OF RENAL CALCULI.

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(*From the Biochemical Laboratory, Cambridge.*)

(With 1 Chart.)

CONTENTS.

Introduction.

The feeding of diets containing excessive amounts of calcium and magnesium was undertaken in a desire to study the effect upon metabolism generally of large amounts of these minerals, and also to observe the effect of each upon the metabolism of the other. It has been shown by various workers that variations in the phosphate content of the diet also affects calcium and magnesium metabolism, but it was felt to be more desirable at first that variations should be restricted to two factors only.

That much magnesium in the diet is a disturbing element in nutrition has been shown by Hart and Steenbock (1913), Haag and Palmer (1928) and by Palmer, Eckles and Schutte (1928–9). The absolute amount of a particular mineral present in a diet is important, but still more important is the *balance* of the various minerals present. Magnesium and calcium have frequently been shown to have antagonistic properties. The trend of observation and experi-

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mentation has been to establish that feeding with large amounts of calcium or magnesium disturbs disadvantageously the storage of the other. Ill effects of overfeeding with either can generally be counterbalanced by a suitably increased intake of the other. There have been a few exceptions to these general findings. Huffman, Robinson, Winter and Larson (1929–30) found no harmful results from giving magnesium carbonate to calves following a diet deficient in calcium. Elmslie and Steenbock (1929), working with rats, found that magnesium given with calcium-deficient diets produced no effects beyond those caused by low food intake and extreme catharsis.

The production of calculi by large doses of magnesium carbonate was so outstanding a feature of the present work that attention was concentrated chiefly upon that. Calculi in rats on a diet deficient in vitamin A have been described by Osborne and Mendel (1917). McCarrison in a series of papers (1926–30) has described several diets which he found led to a high incidence of urinary calculi in rats, and here again the chief and most constant abnormality of the rations was deficiency in the fat-soluble vitamin. Haag and Palmer (1928), during their work on variations on the ratios of magnesium, calcium and phosphorus in the diet of rats, also found calculi in a few instances, but did not study this effect in particular.

In the present investigation many interesting points have not yet been satisfactorily disposed of, but the results up to date are presented in the hope that, though incomplete, they are of some value as a contribution to the study of mineral metabolism in the rat.

EXPERIMENTAL PROCEDURE.

Piebald black and white rats weighing 100–200 grm. were used throughout these experiments. No difference was observed between groups weighing 100–150 grm. and 150–200 grm. respectively. The basal synthetic diet consisted of casein 25, rice starch 40, sugar 17, fat 15, salt mixture 3 parts. Each rat received daily 2 drops of cod-liver oil, and 0·75 c.c. of an extract of marmite (equivalent to 0·75 grm. crude marmite). The salt mixture contained no magnesium or calcium and had the following composition:

Sodium chloride	51·9 parts	Pot. iodide	1·0 parts
Acid sod. phosph.	166·1 ,,	Mang. sulph.	0·2 ,,
Pot. phosph.	386·0 ,,	Sod. fluoride	0·04 ,,
Iron citrate	35·4 ,,		

Calcium and magnesium were added as carbonates. Four diets were used, as shown in Table I.

Table I. *Ca, Mg, and P content of diets.*

Diet		Ca %	Mg %	P %
A	Low Ca/low Mg	0·4	0·2	0·53
B	Low Ca/high Mg	0·4	1·6	0·50
C	High Ca/low Mg	2·0	0·2	0·51
D	High Ca/high Mg	2·0	1·6	0·48

The added amounts of calcium and magnesium (with the exception of the high intake of magnesium, which was twice that used by Haag and Palmer), corresponded approximately with those used by the above workers. The phosphate in the basal diet was not varied, but the final diets had somewhat different percentages owing to the different quantities of added calcium and magnesium. These percentages of phosphorus lie midway between those in Haag and Palmer's "high" and "low" diets.

In the earliest experiments the rats were put on to the experimental diet straight from "stock" diet, consisting of mixed corn, bread and milk, with vegetables or oranges once or twice a week. Distilled drinking water was given to all the experimental animals.

General results.

The animals on diet A continued to grow well and showed no detectable variation from the normal. This was also the case of those receiving diet C. No disadvantageous effects from the extra calcium could be observed, either in general appearance of the animals, growth or post-mortem findings. Diet B was well taken. The daily food intake was not reduced, except for the first few days. The water intake was greatly increased. After a few days on this diet the animals were dull and seemed uncomfortable, but by the end of the first week they were lively and well. Growth was normal, and the general appearance of the rats remained good. Fur was sleek and clean, eyes bright, and when two animals were kept together in a cage, they indulged in normal play. There was no intestinal disturbance, the faeces in fact became dry and chalky in appearance. This condition of well-being continued unless and until some blockage occurred in the urinary tract. Only one rat lost weight; it was the first to develop calculi and was not killed at the onset of symptoms. Thereafter, the animals were carefully watched and anaesthetised as soon as the first sign of discomfort was observed. Each of the eight females used in the first experiment developed calculi in bladder or kidneys. Ureters were sometimes distended, depending upon whether or not there was any actual blockage. Kidney lesions varied in intensity. Sometimes the rats had obviously been killed as soon as bladder calculi were beginning to be formed; in other instances marked kidney lesions were found. Sometimes a kidney consisted of nothing but a thin outer shell of tissue filled with crystalline deposits. In some cases no deposit could be found in the kidney, but hydronephrosis was present. Pain or discomfort only seemed to be caused by a distended bladder due to blockage, so that some animals when killed had only one small calculus, whereas others, apparently healthy and free from pain, were found with extensive kidney damage.

The effect of the diet on the males was not so severe. One rat only, out of the first ten males, had a single calculus and no kidney damage. The remaining males were left for 2 months after it had been found necessary to kill the females, but no abnormalities could be discovered post-mortem.

When placed on the diet the first symptoms appeared after 12 to 44 days (average 21 days) in female rats. On the other hand, the one male which had a calculus had to be killed after only 9 days, and two others in later groups which had only crystalline deposits in the bladder showed discomfort after 17 and 20 days respectively. So that, in spite of the much smaller incidence of trouble in the males, they showed symptoms earlier, and for slighter causes, than did most of the females. There was no relation between the severity of the lesions found in the females and the length of time the animal had been on the diet. The time of onset of the lesions and stone production obviously varied, for one rat killed 44 days from the beginning of the high magnesium intake only had one calculus in the bladder, whereas another rat after 21 days on the diet had severe pyonephrosis.

The effect of balancing the high intake of magnesium with a high intake of calcium (diet D) was striking. Here again, as on diet C, the rats lived in good health for several months and post-mortem findings were negative. There was never the least suggestion of deposits in bladder or kidneys[1].

Further experimental work was mostly confined to a study of the effects of diets B and D. Another similar batch of twelve rats given diet B gave essentially the same results as those described above; all six of the females produced bladder calculi with or without kidney lesions, but in this instance none of the males was affected. A diet containing an equivalent amount of sodium carbonate did not give rise to calculi.

Relation of calculi production to the previous diet of the rat.

It has been pointed out that the above rats were all taken direct from mixed "stock" diet to the synthetic one containing the added amounts of magnesium. This proved to be an important factor. In order to make quite sure of the results previously obtained, a third group was started on diet B. Owing to the former animals having been somewhat uncomfortable for the first few days, with this last batch a slight variation in routine was introduced. For 1 week before being given the low Ca/high Mg or high Ca/high Mg diet, as a preliminary to the more severe régime, they were fed on full synthetic diet similar to that to be given later but containing 5 per cent. of a modified McCollum and Davis (1915) salt mixture, as[2] generally used in this laboratory.

[1] During absence from the laboratory of both the writer and the assistant in charge of the animals, a group of eighteen rats on a low Ca/high Mg diet were given rations which by a misunderstanding contained 16 per cent. magnesium carbonate and 15 per cent. calcium carbonate. This diet contained 2½ times as much magnesium as diet B, yet the animals were quite exceptionally fine and healthy and remained on the diet for 2 months. They were then transferred to stock food; the females later produced normal healthy litters, and none of the rats appeared to suffer in the slightest from their inadvertent drastic dosage with magnesium.

[2] The composition is as follows:

Sodium chloride	51·8 parts	Calcium lactate	390·0 parts
Magnesium sulphate	79·8 ,,	Iron citrate	35·4 ,,
Acid sod. phosph.	104·1 ,,	Pot. iodide	1·00 ,,
Calcium phosphate	162·0 ,,	Mang. sulphate	0·20 ,,
Pot. phosphate	286·0 ,,	Sod. fluoride	0·04 ,,

The reaction of both males and females in this group was quite different from that just described. All the animals of both sexes suffered from more or less severe diarrhoea. Gain in weight was very small and seemed to be due to slight increase in body length, for the rats became very thin, and post-mortem they showed practically no subcutaneous fat. Diarrhoea was sometimes severe, the anus became excoriated and covered with a crystalline deposit. The animals became dirty and untidy in appearance, and presented a striking contrast to the previous groups. Autopsies on the females revealed signs of enteritis, but no bladder calculi or kidney lesions. One male had a single large calculus in the bladder, and another had a large soft blood clot with several calculi embedded in it. The left ureter was distended with blood-stained urine and the left kidney was pale and oedemic. A further group of twelve rats treated similarly gave results in agreement, all the animals suffering from diarrhoea and none showing signs of calculi or kidney trouble. For a long time it was not realised to what these two groups of totally different results were due, but after consideration of every point in connection with feeding and general treatment every possible divergence in routine was eliminated with the exception of the one already mentioned, *i.e.* those rats which had formed white faeces and a high incidence of stone had been taken direct from mixed "stock" diet, whereas those which had diarrhoea and a very small incidence of stone had first been given the normal synthetic diet for a week before receiving the large amounts of added magnesium. (The former of these will, for clearness and brevity, be referred to as "*ex-stock*" and the latter as "*ex-synthetic.*") Altogether over 200 rats, in groups of 12 or 18, have been given the low Ca/high Mg diet after either stock or synthetic food, and the results have been consistent. Diet D (high Ca/high Mg) if given to ex-synthetic rats also resulted in diarrhoea; the calcium had no effect in controlling it.

In attempting to find some explanation of the difference in behaviour of the ex-stock males and females, and the difference shown between these animals and the ex-synthetic ones, an examination of urine, blood and gut *p*H was undertaken.

Effect of previous diet on urinary excretion of Ca, Mg and P[1].

There was no difference in the excretion of magnesium between the ex-stock males and females in the early stages of the experiment. Later on, the amount per diem excreted by the females decreased, and also that excreted by the one male which developed a calculus. Unfortunately, a comparison could not be made between the ex-stock and ex-synthetic animals, as owing to the diarrhoea of the latter it was impossible to collect urine uncontaminated with faeces.

[1] Calcium was estimated by Shohl and Pedley's (1922) method, and phosphate by the Bell-Doisy (1920) colorimetric method. Magnesium was estimated in the filtrate from the calcium precipitate by precipitating it as magnesium-ammonium-phosphate and determining it colorimetrically as phosphate.

The excretion of magnesium by the rats on stock and synthetic diets respectively before they received the added magnesium, revealed some interesting differences. The average daily excretion for males and females separately are given in Table II.

Table II. *Average urinary excretion (mg. per diem).*

	Stock			Synthetic		
	Ca	Mg	P	Ca	Mg	P
Females	0·61	5·80	24·5	2·06	2·75	59·2
Males	0·73	6·60	26·5	1·55	3·02	61·9

The animals were as far as possible similar in weight and age. Thus, the amount of magnesium passing through the kidneys previous to the feeding of diet B was, in the case of the ex-stock ones, twice that excreted by the ex-synthetic ones, while the former were passing one-third to one-half the amount of calcium and approximately half the amount of phosphate excreted by the latter.

Intestinal p*H values.*

The quinhydrone electrode was used throughout for the determination of gut *p*H, and for the first few series the double hydrogen electrode was also used, but proved unsuitable and was discontinued. McRobert (1928–9) and Redman, Willimott and Wokes (1927) have also found the quinhydrone electrode satisfactory in similar work.

The rats were all killed in the early afternoon, food having been left in the cages overnight and removed about 10 a.m. Five regions of the digestive tract were chosen for each investigation: (*a*) upper end of small intestine, immediately leading from the stomach (duodenum); (*b*) lower end of small intestine (ileum); (*c*) caecum; (*d*) colon; (*e*) faeces in rectum or passed during anaesthesia. After death the abdomen was opened as quickly as possible and the technique proceeded with as described in detail by McRobert. In spite of individual variations, rats on the same diet gave curves corresponding very closely. Average figures are given in Table III and curves in Chart I. Each average is the mean of figures obtained from six rats.

Table III. *Intestinal* p*H values.*

	Synthetic diet				Stock diet			
	Males		Females		Males		Females	
	Range	Av.	Range	Av.	Range	Av.	Range	Av.
(*a*)	6·02–6·51	6·26	5·63–6·68	6·19	6·17–6·58	6·37	5·83–6·01	5·92
(*b*)	6·98–7·46	7·22	6·66–6·86	6·74	7·09–7·73	7·43	7·10–7·31	7·20
(*c*)	7·34–7·48	7·56	7·03–7·49	7·26	5·67–6·04	5·83	5·63–5·90	5·76
(*d*)	7·19–7·83	7·72	6·95–7·25	7·10	6·21–6·24	6·22	5·94*	5·94
(*e*)	7·71–7·83	7·72	6·95–7·25	7·10	6·21–6·91	6·50	5·77–6·04	5·90

(*a*) duodenum, (*b*) ileum, (*c*) caecum, (*d*) colon, (*e*) rectum.

* Colon empty except in one rat.

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It will be seen that there was a striking difference between the two sets of rats, the most marked being the acidity of the caecum in the animals on stock diet (average for both sexes taken together *p*H 5·81) compared with that of those on synthetic diet (average for both sexes *p*H 7·28). The figures for stock rats are very close indeed to those obtained by McRobert. He also found acidity of the caecum (*p*H 5·24 average), and the gradually increasing *p*H of the ileum from the stomach to the caecal region. He suggests as an explanation the height above sea-level at which his rats were living (6000 ft.), or lack of excitement, the animals having been killed by a blow on the head. However, the cause is due to neither of these factors, for the rats used in the present work all lived at sea-level and were killed by chloroform anaesthesia.

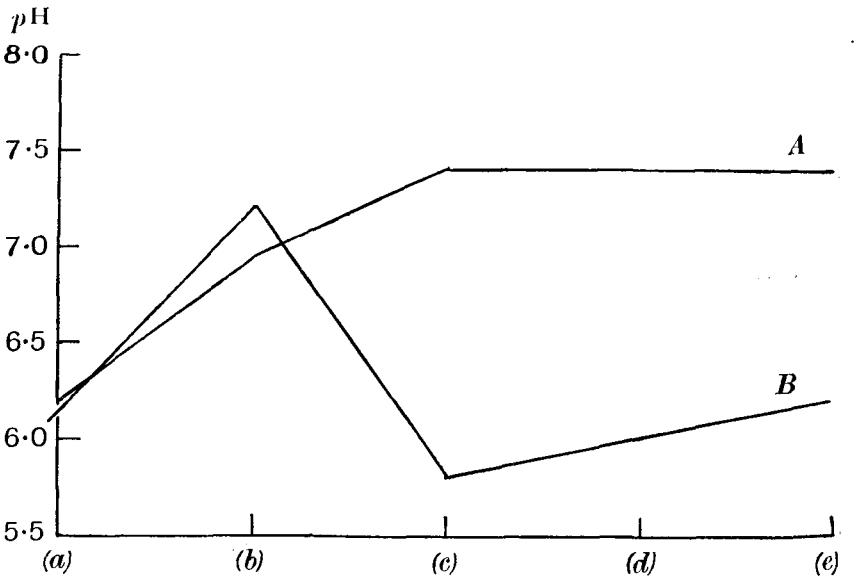

Chart I. Gut *p*H (average for both sexes).

A Synthetic food. *B* Stock food.
(*a*) duodenum, (*b*) ileum, (*c*) caecum, (*d*) colon, (*e*) rectum.

With regard to the rats fed on synthetic diet it will be noticed that not only the faeces but also the colon and caecum were alkaline. According to many workers these are signs of rickets (Zucker and Matzner, 1923–4; Abrahamson and Miller, 1924–5; Redman, Willimot and Wokes, 1927). The latter authors state that on a low fat, rachitogenic diet the caecum, colon and faeces are alkaline (*p*H 7·3). But in the present instance there could be no question of the diet being rachitogenic. The animals were reared on a mixed diet, which we have seen gives no suggestion of rickets in the intestinal *p*H picture, and were only given the synthetic ration for a few days. There was no deficiency of fat in the synthetic food and cod-liver oil was given daily.

The effect was tried of adding the modified McCollum-Davies salt mixture

to stock diet. Mixed corn was ground, mixed with bread, and the salts incorporated at a 5 per cent. level, the whole being made into a paste with milk. The results of four series of estimations are given in Table IV. The rectum of each female was empty. Individual variation in colon and rectum of the males and in the colon of the females was marked, being much greater than was encountered in any of the other rats investigated. The caecum of the females had become definitely alkaline, and that of one male also showed a more alkaline *p*H than any of those on stock diet without the salt mixture. Both females and one male showed increased alkalinity of the colon. Apparently then, at least some of the difference in gut *p*H shown by rats on stock and synthetic diets is due to the difference in salt content. We have already seen that the rats on synthetic diet excrete in the urine (and therefore presumably absorb) twice as much phosphate as do those on stock diet. The significance of these results will be discussed later.

Table IV. *Intestinal* p*H of rats on stock diet and* 5 *per cent. salt mixture.*

	Males		Females	
(*a*)	5·98	6·63	6·80	6·90
(*b*)	7·22	7·42	7·46	7·46
(*c*)	6·80	5·98	7·24	7·09
(*d*)	7·05	5·99	6·90	7·55
(*e*)	7·11	5·99	—	—

(*a*) duodenum, (*b*) ileum, (*c*) caecum, (*d*) colon, (*e*) rectum.

Finally, the gut contents of both ex-stock and ex-synthetic rats on the high magnesium diet were examined. The caecum, colon and rectum were in all cases (males and females, those with, and those without stone) very alkaline. The quinhydrone electrode is unsuitable for measuring *p*H values beyond 8·00, so that no reliance can be placed on the actual figures obtained. In spite of the marked alkalinity of the food containing so high a percentage of carbonate, the reaction of the duodenum was much the same as in the other cases, ranging from *p*H 5·46 to 6·53. In some instances the caecal end of the ileum gave higher values than were obtained with other diets, though others again were normal, the range being from *p*H 6·80 to 7·91.

Serum values of calcium, magnesium and inorganic phosphate.

Many analyses were made of the blood of rats on each of the diets employed, but the results showed no significant variations. Average values are given in Table V, together with the average deviation from the mean.

Table V. *Serum values* (*mg. per* 100 *c.c.*).

	Stock			Synthetic			(*a*) Diet C			(*b*) Diet B			(*c*) Diet D		
	No.	Av.	Av. dev.	No.	Av.	Av. dev.	No.	Av.	Av. dev.	No.	Av.	Av. dev.	No.	Av.	Av. dev.
Ca	5	11·5	±0·8	5	11·7	±0·5	8	12·5	±1·1	7	12·5	±1·1	7	12·8	±1·0
Mg	5	4·8	±0·7	5	3·1	±0·5	8	3·3	±0·2	7	5·2	±0·4	7	5·4	±0·9
P	3	6·1	±1·0	—	—	—	4	5·9	±0·8	6	6·1	±0·8	3	5·3	±1·4

(*a*) high Ca, (*b*) high Mg, (*c*) high Ca and Mg.

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The calcium values were throughout rather high and did not change materially on the various diets, though the males showed increased values on the high Ca/high Mg (D) diet, the average being 13·7 mg. per 100 c.c. Both Richter-Quittner (1924) and Pribyl (1929) have found decreased serum calcium in rabbits after injections of magnesium, but Richter-Quittner used the chloride, and Pribyl's initial values were extraordinarily high, even for rabbits. Injections directly into the blood stream would naturally tend to produce a greater effect than feeding with the same salt.

Phosphates also remained fairly steady throughout, and are normal for the age of rat used. The lowering in inorganic blood phosphate following high magnesium intake found by Palmer, Eckles and Schutte (1928–9) in cattle, is not shown by the rats.

The serum magnesium values of rats on stock diet were higher than for those on synthetic. The difference is considerable, for appreciable changes in serum magnesium are not usually at all easy to induce. The figures given in Table V for diet B are for rats which at the time of death showed no sign of calculi (*i.e.* ex-stock males and ex-synthetic males and females). Much higher values were obtained in those which at the time when blood was taken had calculi or deposits. One animal had 9·33 mg. per 100 c.c. serum. The ex-stock males gave much the same values as the ex-synthetic rats, which it will be remembered suffered from diarrhoea. It appears likely that the high values in the presence of calculi are a result rather than a preliminary to the condition.

Effect on urinary excretion of Ca, Mg and P of high intakes of calcium, magnesium or sodium carbonates.

Although the serum values do not give much evidence of any effect of eating large amounts of calcium or magnesium upon the blood content of either of these elements or upon the phosphate, more interesting results were obtained from the urine examinations. Urine was collected from a number of individual rats for 1 week, this being considered better than following the excretion of a smaller number of animals for a longer period. It is considered unnecessary to give all the figures obtained, the average values and average deviations from the mean only are stated in Table VI.

Table VI. *Average urinary excretion (mg. per diem) on experimental diets.*

Diet	Low Ca/low Mg (A)		Low Ca/high Mg (B)		High Ca/low Mg (C)		High Ca/high Mg (D)	
	Av.	Av. dev.	Av.	Av. dev.	Av.	Av. dev.	Av.	Av. dev.
Ca	1·5	±0·6	0·4	±0·2	1·8	±0·9	3·4	±0·8
Mg	17·4	±0·9	35·2	±4·2	12·1	±9·2	29·1	±6·3
P	50·0	±2·1	10·5	±0·7	15·1	±8·6	5·0	±2·7

The rats receiving large amounts of calcium (diet C) did not excrete any more of this in the urine than the controls (diet A), suggesting that they were not absorbing the salt well. On the other hand, those receiving excessive amounts of both calcium and magnesium (diet D) excreted on an average twice

as much as the controls. It would seem from this that the magnesium helped the absorption of calcium. That these rats on diet D at all events were absorbing some of the extra calcium is borne out by the fact that in the ex-stock animals the addition of calcium to the high magnesium diet prevented the formation of calculi.

The excretion of magnesium became very irregular when calcium was added to the ration (diet C). The average amount in the urine was less than that of the controls, but the deviation was large. The addition of extra calcium to the high magnesium diet hardly affected the magnesium excretion. Many workers have found that giving extra calcium increased the urinary magnesium. Thus, Whelan (1925) found it increased after injections of $CaCl_2$ in the human subject; Mendel and Benedict (1909) found the same result in dogs. On the other hand, Watchorn (1926) failed to find any increase in urinary calcium after ingestion of magnesium acetate.

The animals on diet A which received a little less phosphorus than those on normal synthetic diet (0·53 per cent. compared with 0·66 per cent.) excreted on an average 50 mg. P daily, whereas the addition of large amounts of magnesium to the diet decreased this to 10 mg. daily. It was also diminished by the high calcium ration, and in cases where both salts were added in quantity. Either the extra calcium and magnesium were hindering absorption of phosphate, or larger amounts were being retained to balance the effect of the extra calcium and magnesium. McCarrison (1927–8) states that less phosphate was excreted by the stone-producing animals than by the controls. That the phosphate is being kept back to aid in the formation of calculi cannot be the whole explanation, for a similar decrease in urinary P was shown by the rats on high Ca/low Mg and high Ca/high Mg diets, where no stones were produced. Meigs, Blatherwick and Cary (1910) found that calcium and phosphate were excreted inversely in the urine of cattle, but in the rats used in the present work no special relation was evident between the elimination of these two elements, for the phosphate and the calcium values both fell on adding magnesium to the diet, while the phosphate value fell and the calcium rose when calcium as well as magnesium was added to the food. Orr, Holt, Wilkins and Boone (1924) state that extra calcium given to children diverted some of the phosphate from urine to faeces. As we have seen, increasing the magnesium intake similarly decreases the urinary phosphate, though it is not yet known whether it is diverted to the faeces, or retained in the body, or merely never absorbed. The possible rôle played by the carbonate part of the molecule must not be overlooked. Six rats were given equivalent amounts of sodium carbonate, and in five of them the average amount of phosphate in the urine was reduced to 20 mg. per diem. It is most probable that hindered absorption due to the alkalinity of the diets is the explanation. This may also be the explanation of the decreased urinary calcium associated with diet B, and the apparent poor absorption of calcium in diet C (see Table VI).

Rats receiving large amounts of magnesium excreted only one-quarter as

much calcium as the animals on the control (A) diet. This is not in accordance with the results of other workers (*e.g.* Hart and Steenbock 1913; Whelan 1925; Malcolm 1905); though Pribyl (1929) found the urinary calcium lowered after magnesium injections in rabbits.

It may be considered that in the present work, the calcium values being all small, the differences are insignificant, lying mostly within the limits of experimental error. But it must be remembered that though the figures given in Table VI are given for the *daily* average, the actual estimation was, each time, done on a week's collection of urine, and the differences obtained were well outside the limits of experimental error.

Composition of calculi.

The dried, ground calculi and deposits were analysed by the method described by Newcomb and Ranganathan (1929–30). In Table VII are given the results compared with those obtained by Osborne and Mendel (1917) and by Newcomb and Ranganathan.

Table VII. *Composition of calculi (expressed as percentage of dry weight).*

	Newcomb*	Osborne and Mendel	Watchorn
N	13·3	—	0·1
Ca	1·3	12·1	25·8
Mg	13·3	2·6	16·6
P	24·7	8·9	10·3

* Calculated from actual figures given in original paper.

Newcomb and Ranganathan at first considered that bladder calculi of rats consist chiefly of magnesium-ammonium-phosphate, while the kidney concretions are calcium carbonate and oxalate. Later, however, Ranganathan (1930–31) decided that the apparent oxalate was not, in fact, oxalate at all, but some other permanganate-reducing substance. His analysis of a large number of calculi led him to the conclusion that there was no difference in composition in relation to the site of formation, but that the composition was decided by the diet—that stones rich in calcium were produced on diets to which extra lime had been added, otherwise the concretions were mostly magnesium-ammonium-phosphate.

Reviewed in the light of these results, the composition of the stones and deposits produced by excessive intake of magnesium is extraordinary, for the amount of calcium present was as high as that found by Ranganathan in his "calcium stones," yet the percentage of lime present in the diet was normal. The small amount of ammonia nitrogen may be due to the fact that the stones were not entirely air dried; they were finally dried in an oven at 75–80° C. The amount of magnesium and phosphate present is consistent with the view that the stones were composed largely of magnesium-ammonium-phosphate, for Ranganathan's "calcium stones" contained only traces of magnesium and

very little phosphate (about 1 per cent. P). It is indeed remarkable that so much calcium should be present in stones produced by the agency of magnesium, whereas the magnesium of the stones was only approximately equal to that following diets containing no excess of this ion.

Effect of magnesium on sex organs.

The ex-stock females receiving the low Ca/high Mg diet nearly all had macroscopic abnormalities of the sex organs. The uterus was frequently greatly swollen and filled with blood-stained fluid, even in the absence of cornified cells from the vaginal smear. In any case, the turgidity was much in excess of that normally found at oestrus. The ovaries were often enlarged and the follicles haemorrhagic. In some of the males the seminal vesicles had a cartilaginous consistency instead of the normal softness and flexibility. The changes were not observed in the ex-synthetic rats, and had no relation to the extent of kidney damage or bladder trouble. The males were less frequently affected than the females.

DISCUSSION.

In the various stone-producing diets used by McCarrison, in addition to a deficiency of vitamin A he believes that poor protein and "excess" of phosphate played a significant part. In his 1927–8 paper he gives the amount of phosphate as 1·45 per cent. of P_2O_5, *i.e.* 0·67 per cent. P. This is practically identical with the percentage of inorganic P in the full synthetic diet used in this and many other laboratories, and is less than the P in the whole diet when that present in the casein is taken into consideration. It can therefore hardly be described as excessive and must have had little to do with the etiology of stone production. On the other hand, many of the points emerging from McCarrison's studies have been duplicated in the present work. McCarrison reports that the females were more prone to the disease than the males, and this has also been my experience. Whether or not this is due in part to anatomical differences between the sexes is not known. McCarrison's rats developed hydronephrosis and pyonephrosis in some instances; occasionally there was dilatation of one or both ureters; sometimes he found actual calculi present, sometimes only "gravel." All of these variations have been shown by the females on high intakes of magnesium. Haag and Palmer (1928) had a few instances of calculi occurring in their rats, but, it is interesting to note, only in those on high magnesium/low calcium/low phosphate diets. This is in agreement with the present finding that the addition of extra calcium to the diet prevented the formation of calculi. On the other hand, in the case of the ex-synthetic rats, the diarrhoea was not prevented by the addition of calcium, as it was in the experience of Haag and Palmer. The amount of magnesium used by these authors, however, was only half that used by the writer.

The kidney damage appeared to be caused by back pressure from blockage of a ureter, the magnesium did not cause true nephritis.

The remarkable difference between the ex-stock and ex-synthetic rats is not only interesting but important, inasmuch as it clearly shows what slight variations are required to change completely the resulting picture, and shows how discrepant results may be obtained even when feeding the same diet. The differences, however, are very difficult to explain. In trying to understand what effect the change from stock to synthetic diet for a short period of only a few days could have to lead to such gross differences when magnesium is added later, so that in the one case the gastro-intestinal tract suffers and in the other the renal-urinary system, one thinks at once of a change in absorptive mechanism. We have seen that those animals which later have intestinal disturbances are excreting more calcium and less magnesium than those which later develop calculi. We have also seen that the former have a markedly different gut *p*H from the latter, having an alkaline caecum and colon compared with definite acidity in these regions in the controls on stock diet. This would presumably lead to lessened absorption of salts from the gut, which (in the case of magnesium) would lead to catharsis and intestinal irritation. In the case of the acid caecum enough of the extra salt might be absorbed to produce oversaturation of the urine, with consequent precipitation and stone formation. The increased amounts of phosphate absorbed by the synthetically fed rats might tend to prevent stone formation later, though it is not evident that it would tend to increase the liability to diarrhoea. And it is at least as likely, in the absence of experimental evidence, that the increased amounts of phosphate absorbed might help rather than hinder stone formation by providing phosphate for combination with the magnesium. The results of the gut *p*H investigations might offer a simple explanation of the phenomena if the extra magnesium were fed in the one case as an addition to the stock diet and in the other as an addition to the synthetic diet. But in all cases it was fed to rats receiving the same synthetic diet, the only difference being that previous feeding with the synthetic diet for a few days (1 week at most) prevents stone formation and causes catharsis. Calculi take time to develop, and whatever the differences in the previous diets, whether of salt content or of acidity, one would hardly expect the effect to last sufficiently long to bring about the observed changes. The only explanation offering itself at the moment seems to be that the acid caecum begins at once to absorb much of the extra magnesium, and that the gut wall, having become "sensitised" to this absorption, retains this property even after a change of gut *p*H due to the new diet. This is not in harmony with the views of many investigators, who consider that mineral salts are absorbed in the ileum and partly re-excreted into the caecum and colon. But unless the magnesium is absorbed in the caecum it is impossible to understand why the two sets of rats should not react similarly, since the ileum *p*H is practically identical in both groups.

Elmslie and Steenbock (1929) write that "the digestive tract in its selective absorptive capacity apparently represents an excellent protective mechanism for the exclusion of an excess of magnesium." We have seen that this "excellent

protective mechanism" by no means always functions as such. And even when it does function by keeping the magnesium from entering the system, the condition of the animal is not thereby bettered, for as described by Elmslie and Steenbock themselves, and again shown in the present work, a severe enteritis may develop. In fact, the condition of these animals is considerably the worse of the two, for the others (especially if males) stand a chance of escaping harm, and in any case remain comfortable and lively till near the end of life.

SUMMARY.

1. Rats taken from mixed stock diet and fed synthetic diet containing an excess of magnesium carbonate developed urinary calculi in a large number of cases.

2. The incidence of calculi was much higher in the females.

3. Addition of excess of calcium carbonate to the diet prevented the formation of calculi.

4. Rats previously fed for a short time on the synthetic diet and normal salt mixture did not develop calculi, but suffered from severe catharsis.

5. Rats on mixed stock diet had a more acid caecum and colon than those on synthetic diet.

6. No significant alterations were found in the Ca, Mg or inorganic P of the blood on any of the diets.

7. Extra magnesium, or calcium, or sodium carbonate lowered the urinary phosphate excretion.

8. Extra magnesium or calcium had little effect upon the urinary excretion of the other, but tended in each case to lower the output.

9. Changes in the sex organs brought about by excessive intake of magnesium are described.

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