Relationship Between Prevention of Renal Calcification by Fluoride and Fluoride-induced Diuresis and Reduction of Urinary Phosphorus Excretion in Magnesium-deficient KK Mice

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ABSTRACT Mineral balance studies were performed to clarify the mechanism of the development of renal calcification and its prevention by dietary fluoride (0.1% as NaF) in KK mice fed a low magnesium (0.04%) diet. Upon feeding the diet, the product of urinary calcium and phosphorus concentrations showed a 10-fold increase which was due to a marked rise of the urinary phosphorus concentration. The same phenomenon was also observed in ICR mice which did not develop renal calcification. Therefore, the inherited high susceptibility to renal calcification of KK mice was explicable by a lowered threshold level of the product in the crystal formation of calcium phosphate salt. Supplemental fluoride inhibited the rise of the concentration product, which may partly be responsible for the prevention of the development of renal calcification. The action of fluoride was based on a depressed urinary phosphorus excretion and also a dilution of the excreted calcium and phosphorus by a fluoride-induced polyuria. The diuretic action of fluoride was evidenced by an increased urinary volume, sodium excretion and a decreased osmolality. Feeding the low magnesium diet caused a hyperpotassemia without changes in heart potassium. The hyperpotassemia was prevented by a smaller amount of fluoride than that required for the prevention of renal calcification. J. Nutr. *102:* 893–900, 1972.

INDEXING KEY WORDS fluoride · magnesium · calcification · phosphorus · mice

Magnesium deficiency in KK mice is characterized by much more severe defects with a more acute and reproducible development than those reported in other laboratory animals (1, 2). After feeding a diet low in magnesium and high in phosphorus for only 2 days there occurred severe renal calcification, lowering of glomerular filtration rate, hyperphosphatemia and hyperuremia. These defects were closely related to each other and developed in the described order. Therefore, it was presumed that the renal calcification was a primary cause of the development of the other defects. The cause of the renal calcification, however, has remained an enigma. All these defects were completely prevented following supplementation of the diet with 0.1% of sodium fluoride (3). A partial prevention of deficiency defects by fluoride has been reported on other animal species (4–7), although the mechanism was not clarified.

Mineral balance studies were carried out to clarify the mechanism of the development of renal calcification and its prevention by fluoride. The present report describes the findings on mineral balances of potassium, sodium, calcium, phosphorus and magnesium in KK mice fed the low magnesium diet with or without supplementation of sodium fluoride. A hyperpotassemia which developed in KK mice fed this diet is also described.

MATERIALS AND METHODS

Animals and diets. Specific pathogen-free, female KK[1] and ICR (control) mice

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[1] Mice and stock diet used were those described in the previous report (1).

aged 5 to 6 weeks weighing between 21 and 24 g were used. All animals were weaned at 3 weeks of age and were fed a stock diet. The dietary composition of the stock diet and experimental diets was described previously (1, 2). The only difference between the experimental diets is in their mineral content, which is listed in table 1.

Experiments. Mineral balance was studied as follows. Twelve KK mice were divided in two groups and housed in metabolic cages, each of which contained two mice. They were fed a low magnesium diet with or without a supplement of 0.1% sodium fluoride during 6 days. Twelve ICR mice with approximately equal body weight were used as controls in the same manner. Food consumption was measured daily after drying diets at 40° in a desiccator to eliminate an error due to variable water content. Urine was collected under toluene every 2 days. After measurement of volume, pH and osmolality, the urine samples were made to pH 2 with hydrochloric acid and stored in a freezer. Feces were also collected every 2 days. Urinary and fecal samples were analyzed for potassium, sodium, calcium, phosphorus and magnesium. After 6 days, all mice were killed and the mineral content of tissue and plasma was determined. The experiment was performed twice, and the results were essentially the same. So the data were put together and statistically analyzed by Student's *t* test. Mineral balance was calculated as dietary intake − (fecal excretion + urinary output).

Chemical methods. Mineral salts determinations were made on urine directly and on feces or tissues following ashing with perchloric acid and hydrogen peroxide. Calcium and magnesium were analyzed by atomic absorption spectrometry.[2] Phosphorus was determined by colorimetry (8), potassium and sodium by flame photometry,[3] and osmolality with an osmometer.

RESULTS

1. *Hyperpotassemia induced by feeding a low magnesium diet in KK mice and its prevention by dietary fluoride.* Feeding a low magnesium diet to KK mice for 6 days resulted in hyperpotassemia as well as body weight loss and renal calcification (table 2). The heart potassium in KK mice showed no difference as compared to that

[2] Perkin-Elmer Model 303, Perkin-Elmer Corp., Norwalk, Conn.
[3] Coleman Flame Photometer Model 21, Coleman Instruments Corp., Maywood, Ill.
[4] Fiske Osmometer, Fiske Associates, Inc., Uxbridge, Mass.

TABLE 1

Dietary mineral salt content

Diet		Mineral salt				
No.	Character	K	Na	Ca	P	Mg
				%		
	Stock	0.85	0.31	1.2	0.8	0.27
1	Low Mg	0.61	0.28 [1]	1.2	1.3	0.04
21	High Mg	0.61	0.28	1.2	1.3	0.36
15	Low P	0.61	0.28	1.2	0.5	0.04
	Balanced	0.61	0.28	0.58	0.4	0.04

[1] When diet 1 was supplemented with 0.1% NaF, the sodium content was increased by 0.055%.

TABLE 2

Hyperpotassemia induced by feeding a low magnesium diet to KK mice and its prevention by dietary fluoride

Group	Mice	NaF [1]	Body wt gain	Plasma K	Plasma Na	Heart K	Kidney Ca
		%	*g/6 days*	*mEq/liter*	*mEq/liter*	*mEq/g wet wt*	*mg/g wet wt*
1	KK	0	−1.1 ± 1.2 [2]	6.2 ± 0.5	145 ± 8	75.5 ± 8.7	4.04 ± 0.80
2	KK	0.1	1.5 ± 1.0 [a,3]	4.4 ± 0.3 [a]	143 ± 7	79.8 ± 5.9	0.08 ± 0.01 [a]
3	ICR	0	2.1 ± 1.1 [a]	4.4 ± 0.6 [a]	145 ± 3	81.4 ± 3.1	0.09 ± 0.02 [a]
4	ICR	0.1	1.0 ± 1.5	4.4 ± 0.4 [a]	145 ± 6	81.9 ± 5.9	0.07 ± 0.00 [a]

[1] NaF was added to a low magnesium diet (diet 1).
[2] Mean ± SD of 12 mice.
[3] The superscript a represents $P < 0.01$, when compared with group 1.

in ICR mice. Addition of sodium fluoride to the low magnesium diet completely prevented these changes. In ICR mice none of these changes was observed. The hyperpotassemia was not attributable to a reduction of potassium clearance because potassium excretion in KK mice was not lower than in ICR mice (table 3). Feeding sodium fluoride increased the potassium excretion and decreased balance in both strains of mice, but these changes were not always statistically significant.

An experiment was performed to investigate a relation between hyperpotassemia and renal calcification (table 4). The hyperpotassemia was prevented by feeding the low magnesium diet supplemented with 0.01% sodium fluoride. To prevent the renal calcification, however, a higher supplement of sodium fluoride (0.06%) was needed. Feeding for 4 days a high magnesium diet (diet 21) or a low phosphorus diet (diet 15) which had been known to prevent renal calcification (2) did not induce hyperpotassemia.

2. *Polyuria and enhanced urinary sodium excretion induced by dietary fluoride in KK mice.* Food consumption of KK mice was decreased 2 to 6 days after feeding a low magnesium diet in the balance study (table 5). The decrease was prevented by supplementing the diet with sodium fluoride. The urinary volume in sodium fluoride-treated KK mice was about two times larger than in nontreated mice.[5] The difference in urinary volume was also recognized in ICR mice although the difference in 4 to 6 days was not statistically significant. Urinary pH was slightly but significantly higher in mice fed sodium

[5] Mouse urine generally is highly concentrated as indicated by an unusually high specific gravity and high content of total solids (Biology of the Laboratory Mouse, The Jackson Laboratory, ed., E. L. Green, ed. 2, 1966, p. 344). According to this book, mean urine osmolality of eight strains of mice ranged from 0.61 to 2.63 osmols/kg, and urine output of 12 strains of mice ranged from 0.9 to 3.6 ml/day. Data on ICR or KK mice specifically are not available.

TABLE 3

Urinary excretion and balance of potassium in KK and ICR mice fed a low magnesium diet with or without supplementation of 0.1% NaF [1]

Group	Urinary excretion			Balance		
	0–2 days	2–4 days	4–6 days	0–2 days	2–4 days	4–6 days
	% of intake			*% of intake*		
1	70.9 ± 13.6 [2]	79.1 ± 6.9	71.6 ± 2.2	23.9 ± 12.5	16.0 ± 8.7	25.4 ± 2.3
2	80.1 ± 1.8	78.4 ± 5.6	88.7 ± 7.5 [a,d]	14.1 ± 1.6	18.9 ± 5.6	8.3 ± 8.0 [a,d]
3	67.7 ± 13.6 [d,3]	61.9 ± 5.3 [a,c]	70.0 ± 12.5	28.4 ± 14.9 [d]	35.4 ± 5.8 [a,c]	27.4 ± 13.2
4	84.1 ± 7.7	72.6 ± 2.9	76.8 ± 5.6	9.4 ± 9.1	25.2 ± 3.3	20.8 ± 6.3

[1] This experiment is the same as that of table 2.
[2] Mean ± SD of six samples.
[3] The superscripts a and b represent $P < 0.01$ and < 0.05, respectively, when compared with group 1; c and d represent $P < 0.01$ and < 0.05, when compared with group 4.

TABLE 4

Correlation between renal calcification and hyperpotassemia in KK mice

Diet		No. of mice	Plasma		Kidney Ca
No.	Addition		K	Na	
	%		*mEq/liter*	*mEq/liter*	*mg/g wet wt*
1	0	5	6.0 ± 0.4 [1]	141 ± 2	1.93 ± 1.14
1	NaF 0.0025	5	5.8 ± 0.3	138 ± 3	2.26 ± 1.42
1	NaF 0.01	5	5.1 ± 0.4 [a,2]	141 ± 3	1.00 ± 0.57
1	NaF 0.03	5	4.9 ± 0.5 [a]	140 ± 3	0.22 ± 0.07 [a]
1	NaF 0.06	5	4.9 ± 0.5 [a]	140 ± 2	0.07 ± 0.02 [a]
1	NaF 0.1	5	5.0 ± 0.2 [a]	141 ± 3	0.06 ± 0.01 [a]
1	0	8	6.3 ± 0.3	141 ± 4	4.45 ± 1.03
21 [3]	0	8	4.2 ± 0.7 [a]	140 ± 2	0.06 ± 0.02 [a]
15 [4]	0	8	4.3 ± 0.4 [a]	142 ± 2	0.07 ± 0.01 [a]

[1] Mean ± SD. Mice were fed the experimental diets listed in the first two columns during 4 days.
[2] The superscript a represents $P < 0.01$, when compared with the data on mice fed a low magnesium diet (diet 1).
[3] A high magnesium diet, see table 1.
[4] A low phosphorus diet, see table 1. The only difference of the diet from diet 1 is the phosphorus content: diet 1, 1.3%; diet 15, 0.5%.

TABLE 5

Diet intake, urine volume and urine pH in KK and ICR mice fed a low magnesium diet with or without supplementation of 0.1% NaF [1]

Group	Diet intake			Urine volume			Urine pH
	0–2 days	2–4 days	4–6 days	0–2 days	2–4 days	4–6 days	2–6 days
	g/day/mouse			*ml/day/mouse*			
1	3.6 ± 0.3 [2]	1.7 ± 0.4	1.9 ± 0.5	1.9 ± 0.2	1.7 ± 0.4	1.7 ± 0.4	5.51 ± 0.11 [3]
2	3.3 ± 0.1 [c,4]	3.4 ± 0.3 [a]	3.1 ± 0.2 [a]	3.4 ± 0.4 [a,c]	3.7 ± 0.4 [a]	3.9 ± 0.3 [a]	5.70 ± 0.06 [a,c]
3	4.0 ± 0.1 [c]	3.5 ± 0.3 [a]	3.5 ± 0.2 [a]	1.8 ± 0.2 [d]	2.0 ± 0.4 [c]	2.4 ± 0.7	5.48 ± 0.08 [c]
4	2.5 ± 0.2	3.6 ± 0.2	3.3 ± 0.2	2.3 ± 0.6	3.6 ± 0.6	3.4 ± 0.7	5.57 ± 0.05

[1] This experiment is the same as that of table 2.
[2] Mean ± SD of six samples for diet intake and urine volume data.
[3] Mean ± SD of 12 samples for urine pH data. Urine pH on 0–2 day samples was not measured.
[4] See table 3, footnote 3.

fluoride. Treated KK mice had a higher pH than treated ICR mice. The urinary excretion of sodium was increased and the balance showed a negative value in KK mice throughout the experimental days (table 6). The urine osmolality was also decreased. In ICR mice the effect of fluoride on sodium metabolism was recognized only during the first 2 days.

These results showed a diuretic action of fluoride in KK mice. Another possibility is induction of oliguria by feeding the low magnesium diet. However, as shown in table 7, urinary volume in KK mice fed a synthetic diet with adequate mineral content was not different from that in KK mice listed in table 5. Furthermore, these mice showed normal growth, food consumption and renal calcium content. Therefore it is concluded that feeding the low magnesium diet did not produce oliguria in KK mice.

3. *Urinary calcium and phosphorus concentration, excretion and balance.* The change in urinary phosphorus concentration in KK and ICR mice with time after feeding a low magnesium diet was studied (fig. 1). Before feeding the diet mice were fed a stock diet containing 0.8% phosphorus and had a urinary phosphorus concentration of 120 mg/100 ml. The phosphorus concentration showed a 10-fold increase 12 hours after the feeding. The increase persisted until the termination of the experiment at 48 hours. No strain difference was observed.

The rise in urinary phosphorus concentration was confirmed in the balance study (table 8). The same rise was recognized also in ICR mice. The urinary calcium concentration in KK mice fed the low magnesium diet (table 8) was not significantly different from that in KK mice fed a stock diet (7.9 ± 1.2 mg/100 ml). The product of the calcium and phosphorus concentrations was increased about 10-fold during the first 2 days of feeding the low magnesium diet as compared to the zero time value of 948 (mg/100 ml)^2. Although the marked increase in the product makes a favorable condition for the development of renal calcification, a similar rise also occurred in ICR mice (fig. 1, table 8).

The urinary phosphorus excretion (% of intake) in KK mice fed low magnesium diet was higher than in ICR mice (table 8). The hyperphosphaturia was, however, not a reproducible phenomenon because it was not confirmed in experiments other than that presented in this report. There was no difference in the urinary calcium excretion and balance between both strains of mice (data not shown).

Supplemental fluoride reduced not only the urinary phosphorus but also calcium concentration in KK mice. The product of the calcium and phosphorus concentrations was markedly lowered, especially during the first 2 days of feeding. Again, this decrease in the calcium and phosphorus concentration also occurred in ICR mice. It was found that the urinary phosphorus excretion decreased and the balance increased in KK mice upon feeding the sodium fluoride (table 8). No such effect of fluoride was recognized in ICR mice. The urinary calcium excretion and balance were not altered by supplemental fluoride (data not shown).

4. *Urinary magnesium concentration, excretion and balance.* The urinary magnesium concentration and excretion in KK

TABLE 6

Urinary excretion and balance of sodium and urinary osmolality in KK and ICR mice fed a low magnesium diet with or without supplementation of 0.1% NaF [1]

Group	Excretion			Balance		Osmolality
	0–2 days	2–4 days	4–6 days	0–2 days	2–4 days	4–6 days	2–6 days
		% of intake			*% of intake*		*milliosmols/liter*
1	73.4 ± 9.3 [2]	92.1 ± 5.2	92.1 ± 4.7	19.6 ± 11.2	3.2 ± 5.7	3.3 ± 5.2	2167 ± 298 [3]
2	98.7 ± 3.3 [3,4]	103.8 ± 6.1 [a,c]	102.6 ± 2.9 [a,c]	− 4.8 ± 2.8 [a]	− 6.9 ± 5.6 [b,d]	−5.9 ± 2.9 [b,c]	1292 ± 97 [a]
3	63.9 ± 18.2 [c]	83.7 ± 9.5 [a]	93.1 ± 11.1	32.3 ± 18.0 [c]	13.4 ± 9.8	4.1 ± 11.3	2484 ± 263 [c]
4	97.0 ± 8.7	91.4 ± 5.5	90.5 ± 3.4	− 2.6 ± 9.4	5.6 ± 6.2	6.3 ± 3.4	1506 ± 265

[1] This experiment is the same as that of table 2.
[2] Mean ± SD of six samples for excretion and balance data.
[3] Mean ± SD of 12 samples for osmolality data. Osmolality on 0–2 day samples was not measured.
[4] See table 3, footnote 3.

TABLE 7

General features in KK mice fed a balanced synthetic diet [1]

Body wt gain, g/6 days	2.3 ± 1.0 [2]
Diet, intake, g/day/mouse	3.5 ± 0.2 [3]
Urine volume, ml/day/mouse	1.8 ± 0.2 [3]
pH	5.80 ± 0.04 [3]
Osmolality, milliosmols/liter	2415 ± 198 [3]
Renal Ca, mg/g wet wt	0.07 ± 0.01 [2]

[1] Eight KK mice were fed a balanced synthetic diet (see table 1) in metabolic cages, each of which contained two mice. Diet intake was measured and urine collected every 2 days for 6 successive days.
[2] Mean ± SD of eight mice.
[3] Mean ± SD of 12 samples.

mice were higher than in ICR mice irrespective of sodium fluoride supplement (table 9). The magnesium balance in KK mice was not different from that in ICR mice except for a negative balance in KK mice during 2 to 4 days. The negative magnesium balance was due to a reduced intestinal absorption and an enhanced renal excretion. Feeding sodium fluoride prevented the negative balance.

DISCUSSION

A decrease in muscle potassium and an enhanced urinary potassium excretion without a change in plasma level has been reported in magnesium-deficient rats (9). On the contrary, potassium in heart muscle was increased in magnesium-deficient guinea pigs (7). In KK mice the plasma potassium was increased without a change in the heart muscle potassium. No explanation can be made for the hyperpotassemia. The urinary potassium excretion was increased only between days 2 and 4, so it cannot be concluded that the urinary potassium excretion was enhanced, as in the rat. The hyperpotassemia did not develop upon feeding a low magnesium diet supplemented with sodium fluoride, a high magnesium diet, or a low phosphorus diet. These diets also prevented the development of renal calcification and other magnesium deficiency defects. Therefore the hyperpotassemia may be correlated with the other defects. The hyperpotassemia was not responsible for the renal calcification because the former defect was prevented by a smaller amount of sodium fluoride than the latter.

A high product of urinary calcium and phosphorus observed in KK mice fed a low

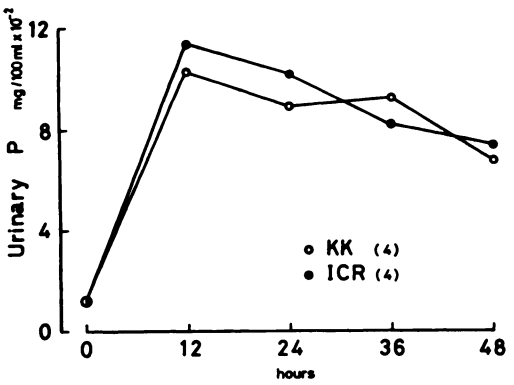

Fig. 1 A rise of urinary phosphorus concentration in KK and ICR mice fed a low magnesium diet. Until zero hour mice had been fed a stock diet whose mineral composition is listed in table 1. The number in parentheses is the number of mice. *At the time indicated, a 20-μl urine sample was collected by pushing the lower abdomen with a finger. The inorganic phosphorus in the urine sample was determined by the method described in the text.*

magnesium diet was brought about by a marked rise in the urinary phosphorus concentration (fig. 1). The rise may be attributable to a low magnesium and high phosphorus content of the experimental diet as compared to a stock diet. This is supported by the fact that the urinary phosphorus was increased with increasing phosphorus intake and decreasing magnesium intake (10).

The high product of the urinary calcium and phosphorus in KK mice may also occur within the lumen of renal tubules, where the calcium deposits were detected. The calcium deposits may be mainly composed of calcium phosphate salts as evidenced by the marked increase in phosphorus content of the kidney. The increase in molar ratio of calcium and phosphorus in the kidney was approximately 2:1. Therefore, the high product of the urinary calcium and phosphorus may make conditions favorable for the development of calcification. The high product was, however, also observed in ICR mice where renal calcification did not develop. Thus the inherited high susceptibility to renal calcification in KK mice was explicable by a lowered threshold level of the product in the crystal formation of calcium phosphate salt. What makes the threshold fall re-

TABLE 8

Urinary calcium and phosphorus in KK and ICR mice fed a low magnesium diet with or without supplementation of 0.1% NaF [1]

Group	Ca concentration		P concentration		Ca × P		P excretion 0–6 days	P balance 0–6 days
	0–2 days	2–6 days	0–2 days	2–6 days	0–2 days	2–6 days		
	mg/100 ml		*mg/100 ml*		*(mg/100 ml)² × 10⁻³*		*% of intake*	
1	10.7 ± 1.6 [2]	5.8 ± 1.7	893 ± 73	639 ± 140	9.6	3.7	42.6 ± 5.9	21.4 ± 8.5
2	4.9 ± 0.7 [a,c]	3.5 ± 1.0 [a,3]	362 ± 15 [a,c]	393 ± 21 [a,c]	1.8	1.4	33.3 ± 4.3 [a]	31.0 ± 8.9 [a]
3	10.0 ± 0.5	5.7 ± 1.6 [c]	905 ± 158 [c]	786 ± 115 [a,c]	9.1	4.5	35.8 ± 4.2 [a]	25.7 ± 6.5
4	8.6 ± 0.6	4.1 ± 0.9	504 ± 52	468 ± 90	4.3	2.0	36.1 ± 4.0	26.9 ± 7.3

[1] This experiment is the same as that of table 2.
[2] Mean ± SD of six samples for the data on 0–2 days, 12 samples on 2–6 days, and 18 samples on 0–6 days.
[3] See table 3, footnote 3.

TABLE 9

Urinary concentration, excretion and balance of magnesium in KK and ICR mice fed a low magnesium diet with or without supplementation of 0.1% NaF [1]

Group	Concentration		Excretion		Balance	
	2–4 days	4–6 days	2–4 days	4–6 days	2–4 days	4–6 days
	mg/100 ml		*% of intake*		*% of intake*	
1	18.7±5.2 [a]	14.8±1.9	46.0±5.0	33.0±2.3	−9.2±10.0	17.1±7.1
2	13.7±0.7 [b,c]	11.9±0.7 [a,c]	37.3±3.6 [a,c]	37.6±2.1 [a,c]	16.3± 6.7 [a]	14.0±4.2
3	9.3±2.9 [a,3]	9.3±4.6 [b]	13.6±4.9 [a]	16.4±5.4 [a]	16.7±10.0 [a]	8.1±8.4
4	6.0±2.3	5.9±2.2	14.4±4.0	15.2±6.1	19.7±15.4	13.6±8.7

[1] Same footnotes as table 3. This experiment is the same as that of table 2.

mains to be clarified. Urinary pH which also influences the development of calcification was not different in KK and ICR mice.

Supplemental fluoride lowered both the urinary calcium and phosphorus concentrations. The lowering of urinary calcium concentration was due to a dilution of excreted calcium by a fluoride-induced polyuria, since dietary sodium fluoride did not reduce the urinary calcium excretion (% of intake). On the other hand, the lowering of urinary phosphorus concentration in KK mice was explicable by a decreased urinary phosphorus excretion as well as the polyuria. The polyuria induced by fluoride was accompanied by an enhanced sodium excretion and a decrease in osmolality. These results were consistent with previous findings that the administration of fluoride caused polyuria in laboratory animals (11, 12). Further, the renal sodium gradient was markedly reduced in the fluoride-induced diuretic rat (13). There has been no report on the effect of fluoride on urinary volume or sodium excretion in magnesium-deficient animals.

Supplemental fluoride reduced a marked rise in the product of urinary calcium and phosphorus concentrations in KK mice fed a low magnesium diet. The reduction was especially pronounced during the initial 2 days, when the renal calcification began to develop, if fluoride was not supplemented. This action of fluoride may partly be responsible for the prevention of renal calcification. Another possible mechanism of the preventive action of fluoride may be an inhibition of the crystal formation of calcium phosphate salts (3, 14). There is also a possibility that fluoride normalized an inherited high susceptibility to calcification with resultant prevention of the calcification. No supporting evidence has, however, been obtained on this point. Fluoride prevented not only renal calcification but also a reduction of growth or a loss of hair in KK mice fed the low magnesium diet. There was evidence that the latter two defects had no causal relationship to the renal calcification (3). Thus, the overall action of fluoride on magnesium deficiency defects in KK mice could not be explained by only the actions on the product of urinary calcium and phosphorus and the crystal formation of calcium salts.

The meaning of a higher urinary magnesium excretion in KK mice than in ICR mice is unclear. The prevention by fluoride of a negative magnesium balance in KK mice fed the low magnesium diet during days 2 to 4 is evidence for a magnesium sparing action of fluoride. Further study should be done to clarify a relationship between this transient action and the preventive action of magnesium deficiency defects.

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