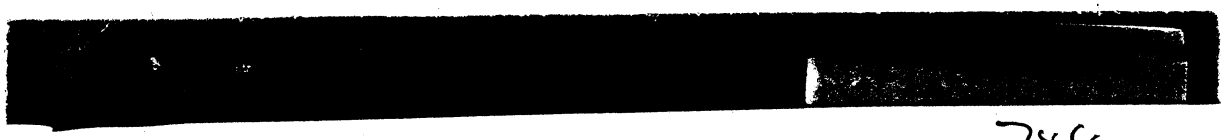

Food, nutrition and chemical toxicity

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CHAPTER 9

Mineral metabolism and chemical toxicity

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Calcium/magnesium homoeostasis, and the role of the kidney in maintaining this, is reviewed. The rat is less able than other species to cope with variation of Ca^{2+}/Mg^{2+} inputs, and this may lead to nephrocalcinosis. Absorption of dietary Ca^{2+} is increased by chemically modified starches, lactose, and various polyols while high-calorie/protein diets cause progressive degeneration of the kidney with loss of mineral homoeostasis, hyperplasia/neoplasia of the parathyroid, mobilisation of Ca^{2+} from bones, metastatic Ca^{2+} deposition, and adrenal medullary hyperplasia/neoplasia. Male rats also exhibit a mineral hydrocarbon nephropathy, because of xenobiotic inhibition of kidney enzymes which hydrolyse $\alpha_{2\mu}$-globulin, a circulating low-molecular-weight protein, that is normally taken up by the proximal renal tubule cells. Resulting tubular damage leads to loss of mineral homoeostasis.

Introduction

Toxicologists involved in the safety evaluation of chemicals are guilty of having taken less interest in the nutritional status of the laboratory animals they study than they should have done, and nutritionists have all too often assumed that maximum growth and freedom from evidence of any form of nutritional deficiency between birth and early adult life, are the only important criteria for defining optimal diets. The possibility that diets too rich in calories and/or essential nutrients might have adverse effects on longevity and risks of disease in the long-term have all too often been overlooked. The only minerals considered in any depth in the present paper are calcium (Ca^{2+}), magnesium (Mg^{2+}), and phosphorus as phosphate (PO_4^{3-}), and most of the text concerns observations in rats, though mice, hamsters, dogs and humans are mentioned here and there. Food is the main source of Ca^{2+}, Mg^{2+} and PO_4^{3-} and it is not only the levels of these minerals in food, but also factors which influence their absorption, that are crucially important on the input side of the equation. In the wild, animals are faced with wide variations in the availability of foodstuffs containing these minerals. Consequently elaborate mechanisms have evolved whereby homoeostasis in tissue, and circulating, mineral levels can be maintained despite wide fluctuations in input. The minerals in question are lost from the body mainly via the urine, but also through secretion and the shedding of cells into the intestinal tract, in sweat, and by the shedding of skin scales, hair, etc. Females lose minerals when menstruating, and during pregnancy and lactation they donate massive amounts to their progeny.

In all species calcium homoeostasis is remarkably, and necessarily, efficient, particularly in relation to the level in blood. Calcium homoeostasis along with that of sodium

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and potassium is, for example, essential for the normal functioning of the heart. The kidney plays a major role in the maintenance of this homoeostasis. The kidney of the laboratory rat, however, is less able than that of most other species to cope with wide variations from optimal inputs of the Ca^{2+}, Mg^{2+} and PO_4^{3-} and failure to cope is manifest, inter alia, as various forms of mineral deposition within the kidney substance and/or within the pelvic space. Mineral deposition in the kidney is commonly referred to as 'nephrocalcinosis'. However, unless deposits seen by the pathologist are positively identified as calcium by special staining, or kidneys are chemically analysed for their mineral content, it is presumptive to assume that calcium is present in the deposits or is the principal mineral present in them.

In toxicity tests on foodstuffs, drugs and other chemicals, agents which interfere with the absorption of minerals, can give rise to disturbances of mineral homoeostasis. An obvious example relates to there being insufficient or too much vitamin D in the diet. Less obvious are dietary ingredients which hamper (eg phytate) or facilitate the absorption of calcium. In the latter category fall chemically modified starches, lactose, various polyols and polydextrose. Rats of many strains are prone — particularly if they are fed on high-calorie or high-protein diets — to develop a progressive degenerative disease of the kidney (nephropathy), which increasingly reduces their capacity to maintain mineral homoeostasis. Failure to do so leads to increased secretion of parathyroid hormone and to hyperplasia of the parathyroid gland. Calcium is mobilised from the bones and the resulting hypercalcaemia leads to metastatic calcium deposition in many different tissues including the kidney itself.

Some of the manifestations of disturbed mineral balance observed in rats seem to carry with them no threat to life. This is true, for instance, for the less severe grades of renal cortico-medullary mineral deposition. However, more major disturbances of mineral metabolism can cause death from renal failure, death from urinary obstruction, inflammation or neoplasia secondary to urinary calculus formation, or death from adrenal medullary neoplasia.

This introduction constitutes the skeleton of this short review. The sections which follow put flesh onto these bones.

Calcium, phosphate and magnesium metabolism

This complex area of science is the subject of many books (eg Nordin, 1976; Kenny, 1981).

The body of a 70 kg man contains about 1.3 kg Ca^{2+} (1.9% of the total weight of the body), 27 g Mg^{2+} (0.04%) and 700 g P (1.0%). Nearly all the Ca^{2+} is located in the skeleton and teeth with only about 8 g in soft tissues, plasma and extra vascular fluid. By contrast, almost half the Mg^{2+} and about 15% of the P is located outside the skeleton. Whereas blood contains only 350 mg Ca^{2+} (in plasma) and 190 mg Mg^{2+} (mainly in red blood cells), it contains 2.0 g of P.

In the UK, a high-milk-consuming country, there is a mean calcium intake of about 1000 mg/day per person. This is about 3 times higher than in low-milk-consuming countries such as India and Japan. The milk of different mammals varies inversely in both protein and calcium content with the time taken to double birth weight. Thus cows' milk has more than twice the concentration of protein found in human milk (3.5% versus 1.6%) and nearly four times the concentration of calcium (125 mg/100 ml versus 33 mg/100 ml). By contrast, human milk contains a higher concentration of the sugar, lactose, which has been shown to promote the absorption of calcium from the gut, in both rats (Fournier, 1965) and humans (Condon et al., 1970; Flatz & Rotthauwe,

1973). Human adults require about 9 mg Ca^{2+}/kg body weight per day. By comparison, the adult laboratory rat, eating, say, 10% of its own body weight of food per day appears to require closer to 500 mg Ca^{2+}/day.

Phosphorus is present in all natural foodstuffs and the daily intakes by humans in different countries vary between 250 and 1100 mg/day with Ca:P ratios ranging from around 0.3 up to around 0.75 in high-milk-consuming countries. In humans very low Ca:P ratios inhibit Ca absorption and very high Ca:P ratios inhibit P absorption. However, in practice the Ca:P ratio is rarely a matter of concern as far as humans are concerned. By contrast Ca:P ratios of 1.0 or higher are required by rats to maintain them in good health in relation to mineral balance (AIN Committee Report, 1977; MRC, 1977; Draper et al., 1972; Phillips et al., 1986).

Like phosphorus, magnesium is widely distributed in natural foodstuffs and dietary magnesium deficiency is not a problem in man. However, in rats, Mg^{2+}-deficient diets predispose to hypercalcaemia and nephrocalcinosis (McIntyre & Davidson, 1958; Newberne & McConnell, 1978). Severe renal failure leads to the retention of Mg^{2+} and hypermagnesaemia. Diabetes is associated with excessive loss of Mg^{2+} in the urine and hypomagnesaemia.

Vitamin D in its various forms facilitates the absorption of calcium from the gut. Unless enough is produced by ultraviolet irradiation (in sunlight) from pro-vitamin D in the skin, there will be a dietary requirement for the pre-formed vitamin. Traces are present in milk, butter and cream, and much larger amounts are present in some fish-liver oils. Dietary vitamin D fortification (eg in margarine) is commonly practised in many Westernised countries. Deficiency of the vitamin leads to osteomalacia and osteoporosis and also to rickets in growing children.

Decline in calcium absorption with age

Duodenal absorption of calcium declines with age, both in rats and in humans. This may reflect no more than the decrease in the absorptive surface area of the gut mucosa which is a feature of increasing age. The decline in middle life arguably matches the lower requirement for calcium after the active, bone-growing phase of life has been completed. However, the continuing decline in humans, especially in women, after the age of 60, because of its association with osteoporosis and hip fracture, seeks a pathological rather than just a physiological explanation. Inadequate dietary intake of vitamin D might be an important contributory cause (Nordin, 1976) although loss of bone matrix associated with post-menopausal hypo-oestrogenaemia is also important.

Intestinal microflora

Reddy et al. (1969) reported that in germ-free rats calcium absorption is much higher than in conventional rats.

Food constituents which affect calcium absorption

The bioavailability of ingested calcium is decreased by phytic acid, oxalic acid and phosphate, but only when large amounts of these substances are present. The absorption of calcium is dependent on an active transport system, and in rats and mice this is particularly highly developed in the duodenum. By contrast, in the hamster it is most highly developed in the terminal ileum (Schachter, 1963; Kimberg et al., 1961). Although it is not a normal site for calcium absorption, active transport has been demonstrated in

the colon of the rat (Harrison & Harrison, 1969). Various investigators have concluded that in both rat and dog the ileum is the principal site of calcium absorption (eg Marcus and Lengeman, 1962; Cramer, 1965).

It has long been known that dietary carbohydrates, and particularly lactose, increase the absorption of calcium from the gut in rats (Bergheim, 1926; Condon et al., 1970; Pansu et al., 1971). Vaughan and Filer (1960) reported that glucose, which normally never reaches beyond the jejunum of the rat, increases the absorption of calcium when introduced directly into the ileum. Recently, we reported that chemically modified starches in high dietary concentrations are associated with increased calcium absorption (Hodgkinson et al., 1982). Fournier et al. (1967) reported that high dietary sorbitol had a similar effect in rats and Pansu et al. (1971) showed, by perfusion studies, that sorbitol can enhance calcium absorption in man.

Drugs which affect calcium absorption

Certain antibiotics, including penicillin and chloramphenicol, have been reported to increase calcium absorption in laboratory animals, probably as a consequence of the modification of gut flora (Wiseman, 1964). In contrast, various anticonvulsants, such as phenobarbitone, have been found to decrease availability (Richens & Rowe, 1970), possibly by inducing enzymes which metabolise vitamin D in the liver (Caspary, 1972). Nordin (1976) lists several other drugs which have been reported to affect calcium metabolism in one way or another.

Factors which affect phosphorus absorption

Most phosphorus is absorbed as inorganic phosphate. The latter may be present in the diet as such or may be formed in the gut after phosphorus has been liberated from phosphosugars, phosphorylated amino acids or phosphonucleotides. However, some organic phosphorus is absorbed in the form of phospholipids. Although the form in which phosphorus is present in the diet influences availability (eg phosphate in the form of cellulose phosphate is only poorly available), in general there is a good correlation between the amount of phosphorus in the food and the amount absorbed. Like calcium, phosphorus is actively secreted into the lumen of the gastrointestinal tract and leaves the body mainly via the urine and faeces.

The duodenum and jejunum are the principal sites of absorption of phosphorus, and the presence of Ca^{2+} ions facilitates its absorption. However, absorption may also occur in the ileum and colon.

Vitamin D enhances the absorption of phosphorus along with that of calcium (Carlson, 1954). It also increases the tubular resorption of phosphate in the kidney (Gekle et al., 1971). Hypervitaminosis D, primary hyperparathyroidism, and genetically-determined idiopathic hypercalciuria, are associated with increased absorption of phosphorus. Aluminium and magnesium antacids bind to phosphorus and impair its absorption (Lotz et al., 1968).

Factors which affect magnesium absorption

Magnesium in foodstuffs is mainly located intracellularly where it is bound to proteins and to phosphate. Chlorophyll, in which magnesium is organically combined in the porphyrin complex, is an important dietary source of magnesium. Digestive processes which break down cell walls are needed to render these forms of magnesium available

for absorption. By contrast, the magnesium present in hard water is more directly available for absorption. Diets rich in phytic acid decrease the absorption of Mg^{2+}. The absorption of Mg^{2+} is concentration-gradient-dependent with no involvement of any active transport mechanism. Magnesium absorption is facilitated by acid pH since it precipitates with phosphate when the pH exceeds 6.5. Notwithstanding these various factors which influence magnesium absorption, there is an approximately linear relationship between dietary Mg^{2+} levels and net Mg^{2+} absorption (Seelig, 1964).

Mg^{2+} may be absorbed throughout the small and large intestine but seems to be greatest in the ileum. No factor which markedly affects the absorption of Mg^{2+} has been identified. However, in humans a high protein diet favours Mg^{2+} absorption (Hunt and Schofield, 1969) and in rats lactose and galactose do so (Forbes, L961; Jacob and Forbes, 1970). Magnesium absorption in rats is increased by certain antibiotics (Heggeness, 1959), and diuretics such as frusemide, which increase the urinary excretion of Mg^{2+}, indirectly give rise to its increased absorption (Nielsen et al., 1969).

Manifestations of disturbed mineral balance encountered in toxicity tests in laboratory rodents

Table 1 lists manifestations of disturbed mineral balance that are most commonly encountered in toxicity studies.

Table 1. Manifestations of disturbed mineral balance commonly encountered in toxicological studies in laboratory rodents.

A	Mineral deposition within the kidney
	1. Cortico-medullary deposits.
	2. Pan-tubular deposits.
	3. Multiple small deposits in the medulla.
	4. Mineral deposits within the pelvic space.
	5. Stone formation within the pelvic space.
	6. Excessive renal calcium as demonstrated by chemical analysis.
B	Acute tubular nephropathy
C	Intra-vesical stone formation
D	Metastatic mineral deposition
	1. Kidney.
	2. Aorta and other large arteries.
	3. Gastric and intestinal mucosa.
	4. Other tissues.
E	Parathyroid hyperplasia and neoplasia
F	Osteodystrophia fibrosa
G	Hypercalcaemia
H	Adrenal medullary hyperplasia and neoplasia

Cortico-medullary and pelvic 'nephrocalcinosis'

Mineral deposition in the cortico-medullary region of the kidney (A.1, see Table 1) and in the pelvic space (A.4) are encountered so commonly in some rat studies that some pathologists in the past have regarded them as no more than variations of normal appearances. However, commonsense suggests that they are pathological in nature though, albeit, usually of minor and non-life-threatening significance. The cortico-medullary mineral deposits may be located within or between tubules while the pelvic deposits may be located under, within, or attached to the luminal side of the pelvic epithelium. Alternatively, they may lie apparently free within the pelvic space near the base of the renal papilla. It is also common for mineral deposits located in this latter position to be associated with adhesions between the epithelium covering the papilla and that lining the outer surface of the pelvic space. In the case of both cortico-medullary and pelvic mineral deposits it is presumptive to regard them as forms of 'nephrocalcinosis' in the absence of positive evidence that calcium is present in them. Nevertheless, the appearances are commonly referred to as 'corticomedullary nephrocalcinosis' and 'pelvic nephrocalcinosis', respectively.

Usually both kinds of deposits are small and unassociated with any inflammatory or other tissue response. However, it is difficult to believe that very large cortico-medullary deposits do not impair renal function. Large pelvic mineral deposits, in the same way as actual stones can be associated with hyperplasia of the transitional epithelium of the renal pelvis and sometimes with chronic and/or acute inflammation . Secondary to these latter changes neoplasms very occasionally arise.

Calculus formation

Actual stone formation within the renal pelvis (A.5) or within the lumen of the urinary bladder (C) are very commonly associated with epithelial hyperplasia and secondary chronic and/or purulent inflammation particularly if a stone blocks a ureter or the urethra. Furthermore, the presence of stones in the lower urinary tract quite clearly predisposes to the development of benign and malignant neoplasms arising in the uroepithelium.

Acute tubular nephropathy

Acute tubular nephropathy (B) is a manifestation of severe disturbance of mineral balance involving, inter alia, excessive uptake of calcium from the gut. It is characterised by the destruction of bunches of adjacent whole nephrons, giving rise to wedge-shaped scars which extend from the outer cortex down to the medulla. These pathological appearances are associated with impaired renal function which can lead to increasing uraemia (azotaemia) and death.

$\alpha 2_{\mu}$-Globulin nephropathy and medullary 'nephrocalcinosis' in male rats

Isolated mineral deposits are not infrequently seen in the renal medulla of older rats. However, multiple deposits associated with tubular degeneration, necrosis and actual disappearance of tubules (A.3) are a feature of $\alpha_{2\mu}$-globulin nephropathy (mineral hydrocarbon nephropathy) — a disease which seemingly affects only male rats. The livers of male rats of up to about one year of age synthesise a low-molecular-weight protein, $\alpha_{2\mu}$-globulin, which finds its way to the kidney via the blood stream. Some of it passes

into the glomerular filtrate from which it is then reabsorbed in the proximal renal tubules. Within the cells lining these tubules the protein is taken up by phagosomes which contain the enzymes necessary to hydrolyse it and thereby release amino acids for recycling or excretion. Chemicals which inhibit the enzymes involved in this catabolic process cause the accumulation of the protein in the phagosomes with the result that cells get progressively larger and eventually die. The dead cells are shed and pass down the tubule to collect in the outer zone of the medulla where they form so-called 'granular casts'. These casts eventually disappear but there remains a functional loss in the damaged convoluted tubules such that they cease to be able to reabsorb calcium and other metallic ions normally. As a consequence, mineral deposits precipitate in the tubules of the renal medulla where reabsorption of water reaches the critical point at which this is bound to happen.

It is not clear to what extent the mineral deposition and destruction of the renal medulla is life-shortening. However, the shedding of proximal renal tubule cells after they have become laden with $\alpha_{2\mu}$-globulin is followed by focal tubular hyperplasia and eventually, in some cases, to the development of adenomas and adenocarcinomas. It is now clear that a wide variety of different chemicals predispose to $\alpha_{2\mu}$-globulin nephropathy in rats and one wonders why the syndrome has only been recognised during the last 15 years. One reason is that, although the granular casts consisting of dead proximal renal tubule cells are pathognomic of the syndrome, they are only present for a brief while and therefore are not found in rats killed terminally in studies of 2 years duration. Also, by the end of a 2-year study, the multifocal tubular hyperplasia which occurs following the destruction of proximal renal tubule cells is difficult to distinguish from the background focal tubular hyperplasia which occurs as part of the chronic progressive nephropathy which afflicts ad-libitum-fed rats, especially males, of most strains. It is however difficult to believe that the multiple medullary mineral deposits which are also pathognomic of $\alpha_{2\mu}$-nephropathy would not have alerted pathologists to the existence of the syndrome had they seen them. In this regard it is relevant to point out that the medullary mineral deposition is seen only if there is an adequate section of the renal papilla. All too often in the past, and even today in some laboratories, the standard operative procedure (SOP) for the preparation of sections of the kidney does not specify that the section should pass longitudinally through the renal papilla from its tip to its base

Disturbances of mineral metabolism associated with chronic progressive nephropathy in rats

The chronic progressive nephropathy to which rats of most strains are prone, especially if they are overfed or given too much protein, is associated with the progressive loss of renal function. One manifestation of this loss of function is failure to conserve (ie reabsorb) calcium from the glomerular filtrate. This failure leads to compensatory hyperplasia of the parathyroid glands (F, see Table 1) and the resulting hypersecretion of parathyroid hormone leads to mobilisation of calcium from the skeleton giving rise to osteodystrophia fibrosa (G). The excessive secretion of parathormone also results in hypercalcaemia (I) and metastatic calcification in various tissues including the kidney itself (D.1), the aorta and other large arteries (D.2), the gastric and intestinal mucosa (D.3), and various other issues (D.4).

Pan-tubular deposition of calcium in the kidney (A.2) is easily distinguishable from the other forms of 'nephrocalcinosis' described above (A.1, A.3, A.4, and A.5). Deposition is liable to affect all parts of the nephron, with the proximal tubules being the

earliest site affected, and the rest of the nephron following as the disease progresses. Parathyroid hyperplasia is an invariable concomitant of this form of nephrocalcinosis and the syndrome is invariably fatal.

Chemical analysis of kidney tissue in the diagnosis of nephrocalcinosis

The approach of the chemist, as distinct from the pathologist, to making a diagnosis of nephrocalcinosis is to analyse kidney tissue by atomic absorption spectrometry for Ca^{2+}, Mg^{2+} and other elements (A.6). This is a relatively straightforward procedure provided that the kidney is exsanguinated following a standard procedure prior to sampling (Hodgkinson et al., 1982).

Adrenal medullary hyperplasia and neoplasia as a consequence of excessive calcium absorption in rats

The rat as a species is especially prone to the development of hyperplastic and neoplastic lesions of the adrenal medulla. Undoubtedly some strains are genetically more suscep-

Table 2. Correlation between metastatic calcification and adrenal medullary hyperplasia/neoplasia among 196 male rats aged 26 months.

		Adrenal medullary hyperplasia/neoplasia	
		–	+
Metastatic calcification (aorta, kidney, lung, etc)	–	55	26
	+	14	24

Significance of positive association $P<0.01$.

Table 3. Correlation between adrenal medullary hyperplasia/neoplasia and incidence/severity of chronic progressive nephropathy in male rats.

	Nephropathy grade*	
	0–2	3–5
Adrenal medulla		
Hyperplasia grade 0–2*	106	48
Hyperplasia grade 3–5 and/or phaeochromocytoma	12	30

$\chi^2 = 20.8$. $P<0.0001$.
*Grade: 0 = none, 1 = minimal, 2 = slight, 3 = moderate, 4 = severe, 5 = very severe.

ible than others while both in genetically-prone and genetically-resistant strains the incidence of proliferative lesions tends to be higher in males than in females.

The observation that dietary ingredients including carbohydrates in general, and lactose, various polyols, and certain chemically-modified starches, in particular, enhance the risk of adrenal medullary proliferative disease, while at the same time increasing the absorption of calcium from the gut and causing various forms of nephrocalcinosis, led to the hypothesis that excessive absorption of calcium is associated with increased incidence of adrenal medullary hyperplasia and neoplasia in rats (Roe and Baer, 1985).

Supportive evidence of two kinds for this theory has subsequently become available. First, in one carcinogenicity study on a non-genotoxic chemical a highly significant ($P<0.01$) correlation was found between metastatic calcification in one or more tissues and adrenal medullary hyperplasia or neoplasia (Roe and Baer, 1985) (Table 2). Secondly, in several other studies, statistically significant correlations between incidence/severity of chronic progressive nephropathy and adrenal medullary hyperplasia/neoplasia have been found (Table 3).

Relationships between increased calcium absorption, nephropathy, hypercalcaemia, metastatic calcification and adrenal medullary proliferative disease in the rat

Figure 1 illustrates these various relationships.

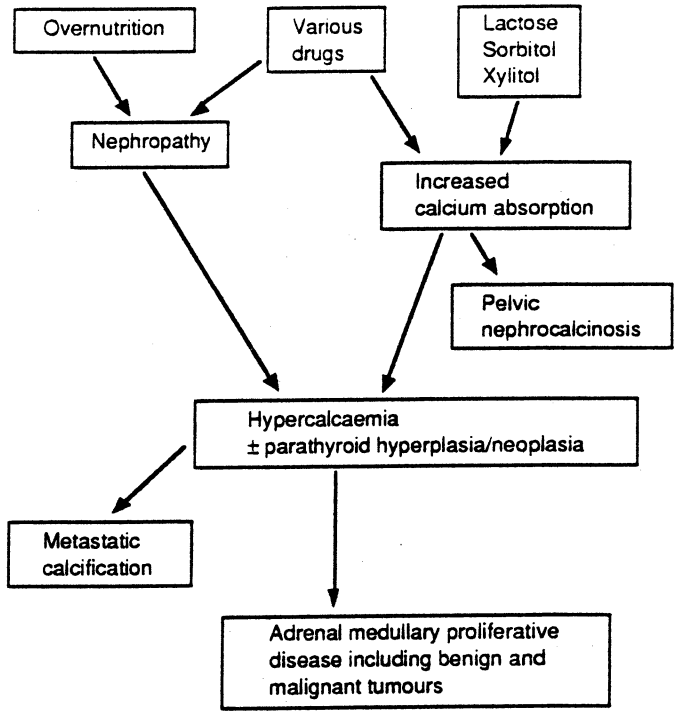

Figure 1. Relationships between increased calcium absorption, nephropathy, hypercalcaemia, metastatic calcification and adrenal medullary proliferative disease in the rat.

Other mechanisms involved in intra-vesical stone formation

Although there is a fairly close association between substances which increase urinary calcium output, pelvic nephrocalcinosis and intra-vesical stone formation, the conditions necessary for bladder stone formation are very often not fulfilled by exposure to substances which give rise to the pelvic nephrocalcinosis. Many variables are seemingly implicated. The formation of crystals critically depends on the concentrations of calcium, phosphate, oxalate etc, and the formation of stones from crystals present in urine critically depends on the presence of proteins and/or cells which serve as a nidus for the start of stone formation. Inflammation of the bladder epithelium facilitates stone formation when other criteria are fulfilled. Rats exposed to high levels (eg 10% in the diet) of the polyol, xylitol, in the diet exhibit increased urinary calcium levels and pelvic nephrocalcinosis but do not develop bladder stones. Similarly fed mice, on the other hand, develop bladder stones in high incidence and also neoplasms arising from the bladder epithelium (Baer, 1987). The reasons for the difference in response are that in mice more xylitol ends up as oxalate in the urine, and that the urine of mice tends to be more concentrated than that of rats. A combination of these factors results in the critical concentration of calcium and oxalate exceeding the solubility limit for calcium oxalate (Baer, 1987).

By contrast, for reasons which have been extensively researched, sugar substitutes such as calcium saccharin in high dietary concentrations (eg 5% or higher) predispose to bladder stone formation and bladder tumours in rats but not in mice. According to Williamson et al. (1987) the probable mechanism responsible involves the stimulation of mitosis in bladder epithelial cells.

A quite different mechanism for bladder stone formation is involved in the case of its occurrence in female rats exposed to the synthetic androgenic steroid, trenbolone acetate. In this case, hormonally stimulated enlargement of the clitoris leads to urinary obstruction and chronic cystitis and the latter predisposes to both stone formation and tumour development (Hunter et al., 1982).

The mechanism whereby lactose and other poorly absorbed carbohydrates increase the absorption of calcium from the gut

Calcium tends to be absorbed along with monosaccharides. Where diets fed to rats contain only normal levels of the most easily absorbed monosaccharide, namely, glucose, then the uptake of this sugar is complete before the food bolus gets much beyond the duodenum. Similarly, simple disaccharides such as sucrose and natural starches, which are readily hydrolysed down to monosaccharides, also do not reach as far as the ileum. In these cases most of the calcium that is going to be absorbed is absorbed in the upper part of the small intestine.

However, if less readily absorbed monosaccharides such as galactose or disaccharides, which give rise to less readily absorbed monosaccharides (eg lactose which breaks down into glucose and galactose), are fed — particularly if they are fed in high amounts — their absorption continues as the food bolus passes through the ileum and possibly even after it reaches the caecum and colon. In these circumstances the absorption of calcium is not confined to the upper part of the small intestine, but takes place over a much greater length of the gut. Furthermore, in the lower ileum the calcium available for absorption is not only that which was present in the food originally, but also calcium secreted into the gut lumen in the succus entericus. In rats and mice the absorption of calcium may be increased many-fold by the inclusion of poorly absorbable carbohydrates in the diet. This theory was amply borne out by Hodgkinson et al.

(1982) in the case of certain chemically modified starches. The introduction of unnatural cross-links and radicals into the starch molecule prevents its hydrolysis in the small intestine, and it is only when the chemically modified starch reaches the caecum where it encounters microorganisms with the enzymes needed for its digestion that it is broken down into absorbable monosaccharides. Like lactose, two different chemically modified starches were found to cause caecal enlargement, increased calcium absorption and many of the pathological changes to which excessive calcium absorption gives rise.

Although the rat as a species has only a very limited capacity to deal with the increased calcium absorption associated with the feeding of poorly digestible carbohydrates, it is possible under extreme conditions to overwhelm the capacity of other species to cope with excessive calcium absorption. Thus, Newberne and Buttolph (1979) encountered acute tubular nephropathy due to hypercalcaemia in hamsters exposed to high dietary concentrations of a highly modified starch. Also, Schach von Wittenau (1981) described a similar syndrome in dogs exposed to a poorly absorbable polydextrose. There is to date no evidence that acute tubular nephropathy can be induced in man by feeding poorly digestible carbohydrates.

Relevance for man

Clearly, humans can tolerate wide variations in intakes of calcium, phosphorus and magnesium and a wide range of calcium:phosphorus ratios without coming to serious harm. By contrast, the laboratory rat is very prone to develop mineral deposition at various sites in the kidney and lower urinary tract if exposed to dietary factors which increase calcium absorption above a tolerated maximum, if the calcium:phosphorus ratio falls below unity, or if the diet is deficient in magnesium. This high susceptibility of the rat to disturbance of mineral balance is coupled with a high susceptibility to chronic progressive nephropathy, (particularly if the diet contains a high level of protein), and with the male-rat-specific renal disease, $\alpha_{2\mu}$-globulin nephropathy. On top of all this is the regrettable inclination on the part of regulatory authorities to require, and of toxicologists to be willing to undertake, toxicity tests in which rats are exposed to grossly unrealistically high dietary levels of substances such as lactose, sorbitol and saccharin. The combination of these factors has led to a galaxy of interesting observations, few or none of which have any obvious relevance to man. Among the galaxy are associations between diets containing high levels of lactose, various polyols or chemically modified starches and the development of hyperplasia and/or neoplasia of the adrenal medulla. In all these cases lower dietary levels have none of these adverse effects. The lack of relevance for man of these observations has been reviewed elsewhere (Roe, 1989). The increased risk of bladder stone formation and bladder tumour development in response to diets containing 5% or more sodium saccharin is also almost certainly of no relevance to humans exposed to far lower doses. Many of the food and other substances which give rise to these disturbances of mineral metabolism in the rat also cause, sometimes quite dramatic, enlargement of the caecum, together with bulky stools or even frank diarrhoea. The only human counterpart of these effects, which invariably quickly disappear on cessation of exposure of rats to the test materials in question, is a tendency to abdominal discomfort and some looseness of the bowels.

Conclusions

The metabolism of calcium, magnesium and phosphorus, particularly that of calcium, is of vital importance to body growth and many bodily functions. This is true for all

species. Man is far more tolerant than the laboratory rat of wide variations in the levels of these minerals in food, and although carbohydrates generally, and lactose and other poorly digestible carbohydrates in particular, enhance calcium absorption in man, the enhancement is small and does not predispose to disease (except perhaps in genetically abnormal individuals, eg hypercalciuric subjects). Studies in rats have played an important role in our understanding the physiology of mineral metabolism. However, they have as often as not 'cried wolf' in tests of food ingredients and xenobiotic chemicals for toxicity. This is partly because of the poor ability of the rat to withstand variations in mineral intake and absorption, and partly because tests have involved exposure to unrealistically high levels of test agents. Apart from this, some of the manifestations of mineral disturbance seen in rats, (eg the association between increased calcium absorption and adrenal proliferative disease), appear to be unique for that species. There are many ways in which test chemicals may directly or indirectly interfere with mineral absorption, mineral homeostatis or mineral excretion. Clues to the occurrence of such interference are provided in the rat by carefully and quantitatively made observations on the incidence/severity of various forms of 'nephrocalcinosis', calculus formation, metastatic calcification and adrenal medullary proliferative changes during the histopathological examination of tissues at the end of tests. These clues can provide valuable information about the mechanism of action of, for instance, drugs. On the other hand, they are not necessarily indicative of toxic risk for man.

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