Perspectives in carbohydrate toxicology with special reference to carcinogenicity

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ABSTRACT

All chemicals, even water and salt, can cause toxic effects if they are given to humans or laboratory animals in high enough doses. Similarly, the incidences of various kinds of neoplasm may be increased non-specifically in animals by the administration of innocent chemicals by an inappropriate route or in doses that are excessive enough to disturb normal nutritional, or hormonal status or interfere with mineral balance.

High dietary concentrations of sorbitol or xylitol, if fed to laboratory rats cause enlargement of the caecum, increased absorption of calcium from the gut, increased urinary excretion of calcium, pelvic and corticomedullary nephrocalcinosis, acute tubular nephropathy, urinary calculus formation and both hyperplasia and neoplasia of the adrenal medulla. High dietary concentrations of lactose give rise to a similar spectrum of effects when given in excessive dosage to laboratory rats. Recent evidence suggesting that in the rat, but not in the mouse or in man, excessive calcium absorption stimulates the adrenal medulla is reviewed.

In the mouse, but not in the rat or in man, a biologically significant amount of glycolic acid, which is a minor metabolite of xylitol in all three species, is converted to oxalate which then appears in the urine. Although the increase in urinary oxalate in the mouse is only about 20% of normal, this is enough in animals fed on diets containing 10% or 20% xylitol to predispose to bladder stone formation, and the prolonged presence of stones in the bladders, particularly of mice, in turn, predisposes to bladder tumour development. Neither bladder stones nor bladder tumours are seen in rats because biologically significant conversion of glycolate to oxalate does not occur. Studies in humans exposed up to 1 g/xylitol/kg body weight/day have revealed no evidence of increased urinary oxalate excretion.

It is concluded that both the bladder tumours seen in mice, in response to 10% or 20% xylitol in the diet, and the adrenal tumours seen in rats, in response to 20% sorbitol or 20% xylitol in the diet, are laboratory artefacts. In other words, humans exposed to "normal" levels of these agents would be at no risk of developing either of these kinds of neoplasm.

INTRODUCTION

The purpose of this paper is to put into perspective for members of the dental profession, the results of long-term studies in which laboratory animals have been fed on diets containing high concentrations of sugar alcohols (polyols). These compounds are of interest because when they are used as sweeteners in food in replacement of sugars, such as sucrose and glucose they do not predispose to dental caries. Indeed they may activily reduce

the risk of caries. However, no-one would wish to prevent caries at the expense of any risk of toxicity or cancer. I, personally, am satisfied that it is completely safe for humans to consume polyol sweeteners both for the purpose of diminishing the risk of dental caries or as alternatives to sugars in the case of diabetes. However, the basis of my opinion is somewhat complex and needs to be presented in several different perspectives.

In the sections that follow I discuss:-

I. The nature of toxicity.
II. The nature of carcinogenicity.
III. The design and interpretation of laboratory tests for toxicity and carcinogenicity.
IV. The effects of over-nutrition in laboratory rodents.
V. How safe is sucrose?
VI. The toxicological evaluation of lactose.
VII. The toxicological evaluation of xylitol and sorbitol.
VIII. Effects of sorbitol and xylitol in humans.

THE NATURE OF TOXICITY

People, including some doctors and, no doubt dentists, who are not professional toxicologists may be tempted to think of chemicals as either (a) toxic or (b) non-toxic. That is to say, they classify chemicals as "black" or "white" with no "greys". By contrast the toxicologist's world consists of various shades of grey with nothing white enough to be advertised as a washing detergent and nothing so black that it could be worn at a funeral. Most of the time the lay press tends to foster the simplistic "black" and "white" view, but I was delighted to see a report in the Daily Telegraph of 14th October, 1983 referring to death from the over-consumption of water (Fig. 1).

In the archives there is a tragic story of the deaths of several new born babies in a maternity unit because a nurse responsible for making up bottle feeds mistakenly added salt instead of sugar. (*Finberg et al.* 1963.)

Clearly both water and salt are essential for life and yet if given in excess they can be killers.

Water and salt 'two of the most innocent poisons'

By CHARLES LAURENCE

WATER, and anything else, can be poisonous if taken to excess. That was the verdict of the medical profession yesterday on the case of the man who drank himself to death with 35 pints of water.

Fig. 1. Headline in London Daily Telegraph of 14th October, 1983.

The toxicologist knows that what is true for salt and water is true for many other chemical agents. An example of interest to the dental profession would be fluoride. In appropriate doses fluoride prevents dental caries, whilst fluoride-deficiency predisposes to caries and excessive exposure to fluoride produces manifestations of toxicity.

THE NATURE OF CARCINOGENICITY

Now what I have just said about water and salt and fluoride is close to common knowledge. It is less well recognised, however, that the same applies to the distinction between "carcinogens" and "non-carcinogens". One cannot categorise all the chemicals in the world simply into "carcinogens" or "non-carcinogens". The situation is far more complex than that.

Take, for example, selenium. This element has been found under different conditions to (a) cause cancer of the liver in rats, and (b) to protect against the development of various other kind of tumour (*Shapiro* 1971, FDA 1972). This indicates that the level of exposure is crucial, with too much or too little being harmful to health. Another recognised example of this relates to the use of antioxidants which are added to prevent rancidity in fatty constituents of foods and drugs, etc. It is widely recognised that oxygen-free radicals predispose to cancerous change and that antioxidants offer protection against the formation of oxygen-free radicals. However, Ito and his colleagues in Japan (*Ito et al.* 1982) found that rats fed on diets containing excessive concentrations of the antioxidant butylated hydroxyanisole (BHA) developed cancers of the forestomach epithelium. The mechanism is not yet clear but, in my opinion, the most likely explanation is that although low concentrations of BHA have an antioxidant action, high concentrations have a pro-oxidant action. If so, then other antioxidants in current use might be carcinogenic if the concentration is elevated into the pro-oxidant range.

In the past, confusion has been caused by the results of research in which animals have been exposed to chemicals by an inappropriate route of administration, as when food ingredients have been given to animals, not by the oral route, but by parenteral injection. In response to daily subcutaneous injections of glucose, 11 out of 47 rats developed sarcomata at the site of its injection (*Nishiyama* 1938).

It is now generally agreed that mechanisms of carcinogenesis fall into two groups: (i) mechanisms involving damage to the genetic content of cells, either to the DNA itself (gene mutation) or to the integrity of chromosomes (clastogenesis) and (ii) non-genotoxic (epigenetic) mechanisms. The latter category includes a wide variety of phenomena, including stimulation of cellular proliferation (tumour promotion), wounding which stimulates repair (wound healing), hormonal effects, and immune-suppression (Fig. 2).

Some agents act directly to cause cancer by either a genotoxic or a non-genotoxic me-

Fig. 2. **Mechanisms of Carcinogenesis**

1.	Genotoxic	gene mutation clastogenesis
2.	Non-genotoxic (epigenetic)	Numerous mechanisms including: (i) tumour promotion (ii) wound healing (iii) hormonal (iv) immune-suppression

chanism. Others act indirectly. For example an agent which is itself incapable of damaging DNA or of interferring with the integrity of chromosomes may be metabolised to an agent which has these abilities. This phenomenon, which is very common, is referred to as "metabolic activation". Non-genotoxic carcinogens may also act indirectly. As an example, I can cite the classical experiment of *Li & Gardner* (1947) in which they implanted one ovary of a mouse into its spleen and removed the other. All the oestrogen produced by the implanted ovary was destroyed when the blood from the spleen passed through the liver and so no oestrogen reached the pituitary gland where, in the normal way, it acts as a signal to inhibit the production of gonadotropic hormone. In the absence of this negative feedback signal the pituitary hypertrophied to produce more and more gonadotrophic hormone. This in turn stimulated the ovary (in the spleen) to produce more and more oestrogen. The final outcome was that the mice in question developed gonadotrophin-producing pituitary tumours, oestrogen-producing ovarian tumours, and marked toxic changes (peliosis hepatis) in the liver.

As I will explain, a range of tests has been developed to evaluate chemicals for gene-mutagenicity, clastogenicity and various forms of non-genotoxic carcinogenicity. However, the results of such tests have to be carefully evaluated because false positive and false negative results are not uncommon. In the case of long-term tests in living animals, unnatural aspects of laboratory life predispose to high incidences of tumours in untreated control animals and this noisy background, in particular, is a source of problems in interpretation.

THE DESIGN AND INTERPRETATION OF LABORATORY TESTS FOR TOXICITY AND CARCINOGENICITY

In medieval times, the King is said to have tried out foods on dogs or servants before partaking himself, just to make sure that he was not being poisoned. Unfortunately such rough and insensitive methods could not be relied upon to pick up insidious toxins and potential carcinogens. By contrast the main problem of the testing systems we use today is arguably their over-sensitivity and propensity to give misleadingly positive results.

Usually the first step today is to test a new chemical, such as a prospective food additive, drug or pesticide for genotoxic activity. For this purpose there now exist a number of well validated tests both for gene mutagenicity and clastogenicity. The cheaper and quicker tests cam be carried out in test tubes, on bacteria or on mammalian cells maintained in tissue culture. To ensure that indirectly acting genotoxins are recognised tests involving the addition of metabolising enzymes are normally included. If any doubt remains regarding possible genotoxicity, then *in vivo* tests for gene mutation and clastogenicity may be required.

It is unlikely that any agent which gives negative results in a selection of these tests for gene mutagenicity and clastogenicity could act as a genotoxic carcinogen in man. However, an agent capable of producing tumours by a non-genotoxic mechanism might consistently give negative results in such tests, and at present, it is inconceivable that one could devise a series of simple short-term and inexpensive tests which would reliably exclude all varieties of non-genotoxic carcinogenicity. For this purpose it is necessary to carry out experiments in which laboratory animals are exposed for prolonged periods to test chemicals by an appropriate route of exposure. In the case of a food constituent or a food additive, the appropriate route is orally, either by admixture with the food or by gavage.

The usual practice is to design an experiment in which different groups each of, say, 50 male and 50 female rats or mice are exposed to the test agent at various multiples of the dosage rate for humans. For a minor food additive or for a pesticide residue, it may be

possible to test the response of animals to doses of a chemical that are, say, 100, 1,000 or even 10,000 times higher than the level of human exposure. Obviously, negative results for toxicity and carcinogenicity at all dose levels in such tests provide very considerable reassurance of safety for man. However, for a major food ingredient, e.g. a substance that constitutes say 1% or 5% or more of the diet, it is not possible, *without distorting the nutrient composition of the diet,* to assess the response of animals to levels of the substance that materially exceed those to which man may be exposed. Furthermore, if longterm animal feeding studies involving gross dietary nutrient distortion are undertaken, the results are likely to be difficult to interpret. This is, as I shall explain, exactly what has happened in the case of certain long-term studies in rodents on lactose, xylitol and sorbitol.

In practice one would reject for food use an agent which is, or is likely to be, genotoxic under realistic conditions of exposure. The problem is to know what to do about non-genotoxic agents which, *under unrealistic conditions of exposure,* have been found to give rise to evidence of toxicity or carcinogenicity in laboratory animals. It is *not an option to ban all such substances* since we cannot ban sucrose, glucose, lactose, selenium, antioxidants and orange juice (*Field & Roe* 1965) etc.

THE EFFECTS OF OVERNUTRITION IN LABORATORY RODENTS

Before I consider the toxicological profiles of particular sugars and polyols, I need to discuss yet another perspective of long-term toxicity testing in animals, particularly since it is very relevant to the interpretation of the results of animal studies with sucrose.

It is the usual custom in the conduct of long-term toxicity and carcinogenicity studies in rodents for animals to have free access to food throughout the 24 hours of each day. If this is done, and if the diet provided is overnutritious particularly in respect of fat, then the animals become very obese and develop a variety of pathological conditions (*Roe* 1981). In the case of rats a progressive form of chronic nephropathy, affecting both the glomeruli and the tubules, and high incidences of endocrine tumours, including pituitary and mammary tumours, are some of the hallmarks of overfeeding (Table 1). Simple diet restriction as by removing the food basket from the cage for a part of each day, or by rationing the amount of food available, may dramatically prolong life, prevent the nephropathy and reduce tumour incidence (Figs. 3 and 4, Table 2).

Similarly, dramatic effects on longevity and tumour incidence may be achieved by diet restriction in mice although, in this case, the principle kinds of tumour affected are malig-

Table 2. Effects of time-restricted access to food in Wistar rats fed on a standard diet.

Hours of access per day	24		6.5	
Sex	♂	♀	♂	♀
% survival for 2 years	50	70	90	80
% with moderate or severe nephropathy	65	60	5	0
% with pituitary tumour	30	55	20	25
% with mammary tumour	-	10	-	0

Table 1. Effect of dietary restriction on incidence of pituitary and mammary tumours in rats (*Tucker* 1979).

Feeding regimen . . .	Males		Females	
	Ad lib.	Restricted	Ad lib.	Restricted
Rats with pituitary tumours (%)	32	0***	66	39**
Rats with mammary tumours (%)	0	0	34	6***

** = $p < 0.01$ *** = $p < 0.001$.

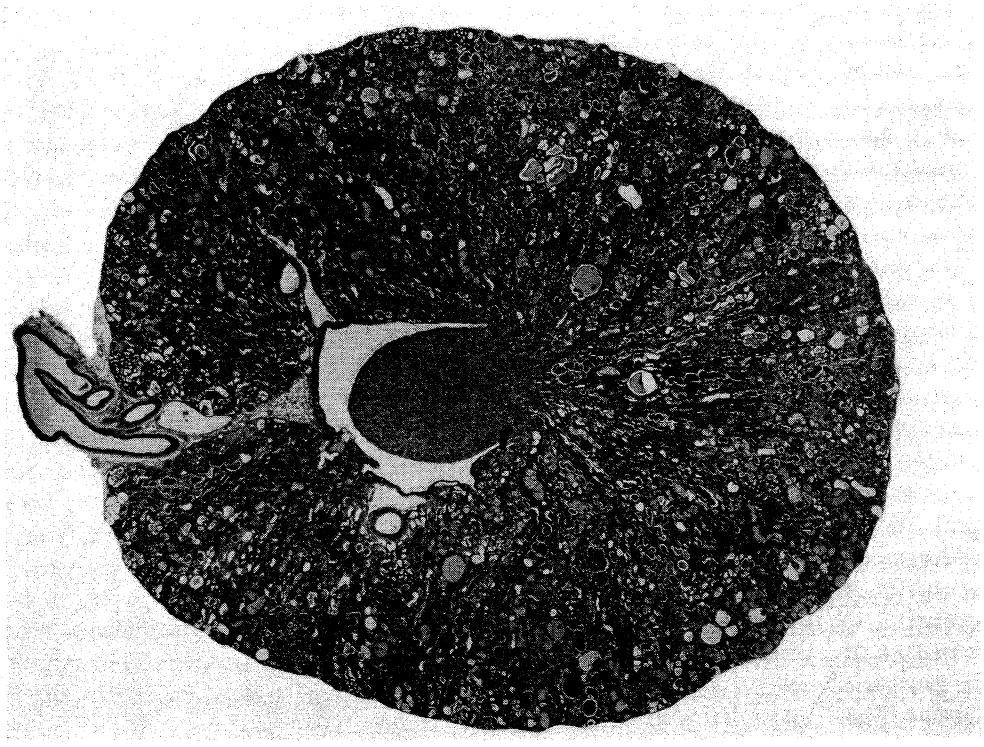

Fig. 3. Kidney of a 25 month old male Wistar rat given free access to a standard laboratory diet throughout the 24 hours of each day.

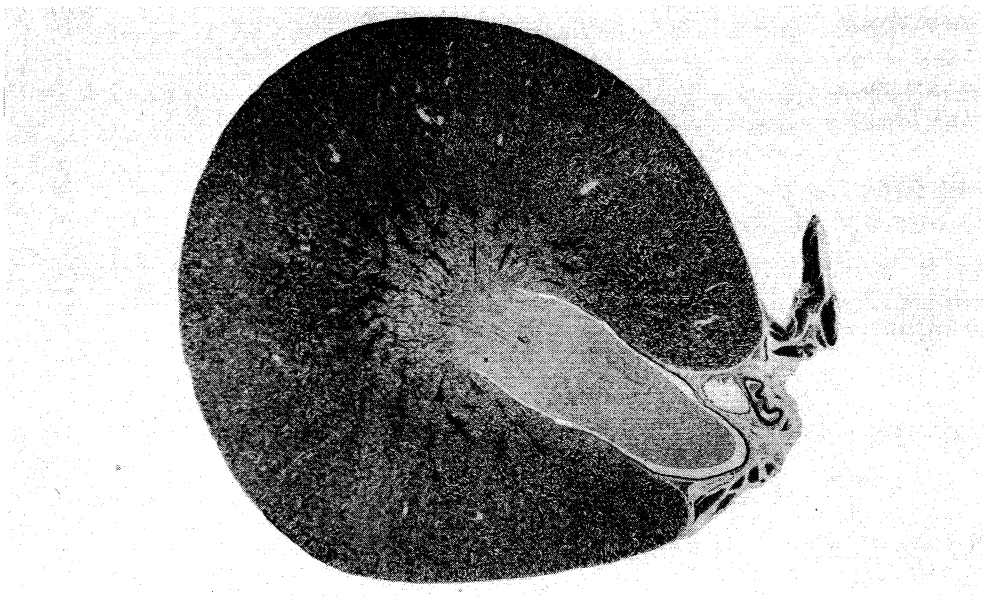

Fig. 4. Kidney of a 25 month old male Wistar rat given free access to the same standard diet (see Fig. 3) but for only 6½ hours each day.

nant lymphoma, liver and lung tumours (*Tucker* 1979 and Table 3).

One may conclude from data such as these that the results of carcinogenicity tests may be difficult to interpret if exposure to the test substance disturbs the nutritional status of animals. Furthermore, in the case of rats it is clear that overnutrition leads to serious disturbances of hormonal status and to impairment of renal function.

HOW SAFE IS SUCROSE?

Sucrose is, of course, not genotoxic. In humans, a high sucrose diet predisposes to obesity and all the medical complications of obesity. A high sucrose diet also predisposes to diabetes mellitus and to dental caries. All these effects occur also in long-term feeding tests in laboratory animals.

In 1949, *Best,* one of the discoverers of insulin, and his colleagues reported that rats exposed to 15% ethanol in their drinking water while being fed *ad libitum* on a standard laboratory diet developed fatty livers. But sucrose in isocaloric amounts had the same kind of effect in even greater degree! (*Best et al.* 1949.)

Since over-nutrition is associated with increased cancer risk in rats and mice, it is not surprising that in mice, high sucrose diets have been found to be associated with increased liver tumour , see Table 4 (*Hunter et al,* 1978 a).

Thus excessive doses of sucrose, like excessive doses of anything else, are potentially hazardous to health.

RESPONSE OF RATS TO LACTOSE IN HIGH ORAL DOSAGE

The milk of mammals contains the disaccharide, lactose, as its carbohydrate component. Within the gastro-intestinal tract of the suckling infant, lactose is split by the enzyme, lactase, into galactose and glucose. A genetically-determined absence of lactase from the gut predisposes to milk intolerance. In lactase-deficient individuals, lactose *per se* reaches the large intestine where it may to some extent be broken down by lactase of bacterial origin. There is a tendancy among some humans who are adequately endowed with lactase as infants to become lactase-deficient as they grow older. Such individuals become progressively more and more intolerant of milk.

The results of much research in the past (e.g. *Vaughan & Filer* 1960, *Dupuis & Fournier* 1963) have suggested that calcium is absorbed from the gut along with monosaccharides and that the facilitation of calcium absorption is greater for some monosaccharides

Table 4. Effect of dietary sucrose for 100 weeks on liver tumours incidence in female CFLP mice (*Hunter et al.* 1978).

	Control	20% sucrose
No. of mice	87	89
No. that developed liver tumours	15	31*

* $p < 0.05$.

Table 3. Effect of diet restriction on longevity and tumour incidence in mice (*Conybeare* 1980).

	Ad lib.	Restricted (75% of ad lib.)	Ad lib.	Restricted (75% of ad lib.)
Number of mice	160	160	160	160
% survival to 83 weeks	58	66	62	77*
% lung tumours	19	12*	15	5**
% liver tumours	29	8***	4	0.6*
% any tumour at any site	44	23***	31	11**
% any malignant tumour at any site	11	4*	14	4**

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

than for others. Galactose is one of the sugars that is particularly effective. It is not certain why sugars differ in this effect, but it probably relates to the ease with which they themselves are absorbed. Glucose is so readily absorbed that when given in reasonable quantities by mouth, absorption is complete within the stomach and duodenum. By contrast, after the administration of similar amounts of galactose, some of the sugar may still be unabsorbed at the time the bolus of food reaches the jejunum, ileum or even large intestine. In these circumstances the proportion of the gut in which calcium is being actively absorbed may be greatly increased with the result that *more calcium is absorbed. Fournier et al.* (1965) is among those who have postulated that this effect is beneficial for the infant mammal which needs to achieve positive calcium balance during the bone-forming stage of its development. A corollary to this may be that increasing lactase deficiency with age protects against the absorption of too much calcium when bone-growth is complete. *Cochet et al's* (1983) recent report that lactose enhances calcium absorption in lactase-competent humans but reduces it in lactase-deficient individuals is consistent with the theory that it is not lactose itself which influences calcium absorption, but galactose.

When rats are fed on diets containing lactose in excessive amounts, several effects are seen depending on the extent of the overloading (Table 5).

All these effects in rats with the possible exception of those on the adrenal medulla are interlinked. Caecal enlargement occurs because the capacity of the rat to break lactose down to absorbable monosaccharides within the first part of the small intestine is overwhelmed by a 20% level of incorporation in the diet. Calcium absorption and urinary excretion are increased because galactose absorption is proceeding over a much longer length of the gut than normal. The various pathological changes in the kidney (pelvic and cortico-medullary nephrocalcinosis; acute tubular nephropathy) are secondary to the increased calcium absorption. The only uncertainty relates to the pathogenesis of the proliferative changes in the adrenal medulla.

The effects of 20% dietary lactose on the adrenal medulla are depicted in Table 6.

Calcium is known to play a role in the release of catecholamines from adrenal medullary chromaffin cells (*Perlman & Chalfie* 1977). *Brion & Dupuis* (1980) reported that low blood calcium levels in vitamin D-deficient

Table 5. Effects of excessive dietary lactose in rats. [References: *Hodgkinson et al.* 1982, *de Groot & Feron* 1975/76, *Vaughan & Filer* 1960 and unpublished data (adrenal).]

Tissue	Effect
Caecum	Enlargement
	Loose stools
Intestine	Increased calcium absorption
Kidney	Increased urinary calcium
	Pelvic nephrocalcinosis
	Cortico-medullary nephrocalcinosis
	Acute tubular nephropathy
Adrenal	Hyperplasia
	Benign and malignant neoplasia

Table 6. Effect of 20% dietary lactose on adrenal medulla in rats. Details and an acknowledgement of the source of these data will be given in a forthcoming publication (*Roe & Bar*).

% rats with	♂		♀	
	Control	20% Lactose	Control	20% Lactose
Hyperplasia or phaeochromocytoma	41	71	16	26
Phaeochromocytoma	23	44	2	4
Malignant phaeochromocytoma	7	20	0	2

rats could be restored to normal simply by adding lactose to the feed. Furthermore, with the restoration of the serum calcium levels to normal, the depression of adrenal medullary function, which is a feature of the vitamin D-deficient state, was also restored to normal. These observations tempt one to speculate that excessive absorption of calcium predisposes to *hyperfunctioning* of the adrenal medulla with associated hyperplasia and eventually neoplasia. However, this is clearly oversimplistic in-so-far as the hyperplastic and neoplastic adrenal medullary cells in lactose-fed rats are chromaffin negative and there is no evidence that the phaeochromocytomas in these animals are associated with vascular hypertension, increased dopamine release or increased urinary excretion of catecholamine metabolites.

Non-functional (chromaffin-negative) proliferative lesions of the adrenal medulla are, for some unknown reason, quite common in untreated laboratory rats, particularly in males (*Roe* 1983). Incidences as high as 51% (*Kociba et al.* 1979) and 81% (*Gillman et al.* 1953) have been described in different strains. Such lesions in such high incidence have no counterpart in man.

THE RESULTS OF LABORATORY TESTS WITH XYLITOL AND SORBITOL

Like lactose, these two polyols are not genotoxic. In a long-term study in rats conducted at the Huntingdon Research Centre (*Hunter et al.* 1978 b) 50 male and 50 female Sprague-Dawley rats were exposed to 2, 5, 10 or 20% xylitol, 20% sorbitol or 20% sucrose in the diet. A similar sized group of control rats received a control diet of which 20% was rice starch. In the test diets xylitol, sorbitol or sucrose replaced rice starch. At a 20% level of incorporation of sorbitol or xylitol in the diet, rats received doses of about 7.5 g/kg body weight per day. This dose rate is equivalent to a 70 kg man consuming over ½ kg of the polyol each day. The spectrum of effects seen in the rats receiving 20% xylitol o 20% sorbitol was identical to that reported for rats exposed to a diet containing 20% lactose (*Hodgkinson et al.* 1982). The list of effects included:-

Reduced body weight gain
Caecal enlargement
Increased water consumption
Increased urinary output
Pelvic nephrocalcinosis
Proliferative lesions of the adrenal medulla

The sections of the adrenal gland were reviewed by *Russfield* (1981) who reported the incidence of adrenal medullary lesions depicted in Table 7.

The 20% sucrose diet was associated with lower incidences of both hyperplastic and neoplastic lesions of the adrenal medulla compared with the rice starch controls, but the differences were not statistically significant.

Table 7. % rats with adrenal changes after exposure to xylitol or sorbitol for 79 or more weeks. [*Russfield* (1981) re-evaluation of *Hunter et al.* (1978).]

	Control	20% Xylitol	20% Sorbitol	20% Sucrose
Males				
Medullary hyperplasia	10	29	38**	6
Phaeochromocytoma	17	31	13	6
Females				
Medullary hyperplasia	5	25**	17	3
Phaeochromocytoma	2	14	11	8

** $p < 0.01$.

As in the case of lactose, it is reasonable to postulate that the enhancing effect of xylitol and sorbitol on calcium absorption is in some way implicated in the effect of these polyols on the adrenal medulla. Recently *Dr. Albert Bär* (personal communication) investigated the effect in rats of a 20% xylitol diet which contained 0.4, 0.2, 0.1 or 0.05% calcium. A control group were given a diet containing 20% starch and 0.4% calcium. The extent of caecal enlargement in the xylitol group was not affected by the level of calcium in the diet. However, there were differences between the groups in adrenal adrenalin and dopamine levels. These were significantly higher in the rats receiving 20% xylitol and 0.4% calcium in the diet than in rats receiving starch and the same level of dietary calcium. This effect of xylitol was abolished by reducing the dietary level of calcium to 0.1% or to 0.05%. These data provide persuasive evidence that the adrenal pathology seen in xylitol-treated rats is secondary to the enhancement of calcium absorption by xylitol.

In another study (*Hunter et al.* 1978a), groups of 100 male and 100 female CFLP Swiss albino mice were exposed to diets containing 2, 10 or 20% xylitol of 20% sucrose. Controls were fed the same rice-starch containing diet as the controls in the rat study. It was in this study that a significant excess of liver tumours was seen in females fed on the 20% sucrose diet. No treatment-related changes were observed in the adrenal glands in any group. However, male, but not female, mice in the 10% and 20% xylitol groups developed high incidences of bladder calculi, associated with which there occurred epithelial hyperplasia and, in some cases, tumours of the bladder epithelium. With only one exception, all the mice that developed bladder tumours also had bladder stones at the time of necropsy (Table 8). There is plenty of published evidence showing that the presence of stones predisposes non-specifically to bladder

Table 9. Tumour incidence in male mice.

	C	20S	2X	10X	20X
No. of mice necropsied	90	94	90	92	92
Lympho-reticular	22	29	26	27	20
Lung	33	38	41	30	30
Liver	47	48	37	30*	29*
Kidney	5	11	2	3	1
Bladder	0	0	0	9**	11**
Testis	11	7	6	5	8
ANY TUMOUR	77	80	72	70	60

* = $p < 0.05$ ** = $p < 0.01$.

Key
C = Control diet
20S = 20% sucrose in diet
2X, 10X and 20X = 2%, 10% and 20% xylitol in diet respectively

Table 8. Changes in the Urinary Bladder of Males.

	Examined macro.	Stones present	Examined micro.	Epithelial hyperplasia	Epithelial metaplasia	Any tumour	Malignant tumour
C	90	2	77	12	0	0	0
20S	93	3	87	11	0	0	0
2X	91	2	81	10	0	0	0
10X	92	58	85	60	5	9*	3*
20X	92	70	86	63	10	11	5

* One mouse in the 10X Group had a malignant tumour but no stones at the time of necropsy. All other mice which had tumours also had stones.

Key C = Control diet
20S = 20% sucrose in diet
2X, 10X and 20X = 2%, 10% and 20% xylitol in diet respectively

tumour development in mice (*Ball et al.* 1961) and this is undoubtedly the explanation in the present case. It is noteworthy that neither stones nor bladder tumours were seen in males in response to the 2% xylitol diet, that no stones or tumours were seen in females at any dietary concentration of xylitol, and that there was no excess incidence of any kind of neoplasia at any other site in males or females (Tables 9 and 10). In fact, at both the 10% and 20% level, significantly fewer xylitol-treated male mice developed liver tumours than control males.

The bladder stones in these xylitol-treated mice consisted of calcium oxalate. The fact that xylitol increases the absorption and urinary excretion of calcium is clearly a contributory factor to the stone production. However, it is not a sufficient explanation since high dietary lactose, which has the same effects on calcium absorption and urinary calcium only gives rise calculi in mice of administration is in very high concentration in the diet (*Feron et al.* 1978). Another factor must be involved. This has been identified as a species effect of xylitol in increasing glycolic acid and oxalate production in mice. According to *Robertson et al.* (1978), because calcium oxalate is only poorly soluble in water, even a small increase in urinary oxalate materially increase the risk of bladder stone formation. Although this effect of increased oxalate is magnified further by high urinary calcium levels the effect of high calcium *per se* is comparatively small.

Table 10. Tumour incidence in female mice.

	C	20S	2X	10X	20X
No. of mice necropsied	87	89	89	86	88
Lympho-reticular	32	29	34	30	28
Lung	20	26	21	23	18
Liver	15	31*	18	19	17
Bladder	0	0	0	0	0
Overay	5	5	3	4	7
ANY TUMOUR	62	66	64	60	59

* = $p < 0.05$.

Key

C = Control diet
20S = 20% sucrose in diet
2X, 10X and 20X = 2%, 10% and 20% xylitol in diet respectively

In the mouse the effect of xylitol on urinary oxalate excretion is itself not very great (e.g. only a 20% increase). Nevertheless, this is enough to predispose to stone formation.

The fact that a 20% xylitol diet does not enhance urinary oxalate excretion in rats (*Salminen et al.* 1983 a), explains why bladder stone formation is not a consequence of high dietary exposure to xylitol in this species even though such exposure is associated with increased urinary calcium levels. *Salminen et al.* (1983 b) reported another difference between the rat and the mouse. After a period of dietary exposure to xylitol, mice of two different strains were found to absorb more oxalate from the gut than control mice. By contrast rats did not show this adaptive effect.

EFFECTS OF SORBITOL AND XYLITOL IN HUMANS

Experiments involving the exposure of laboratory rats and mice to diets containing 10% or 20% sorbitol continuously through life clearly do not meaningfully imitate conditions of human exposure to these polyols. For this reason alone the effects of these high dietary levels of xylitol on the bladders of mice may be dismissed as laboratory artefacts. Apart from this, however, it is now clear that mice, particularly the males of some strains, respond in a peculiar way to xylitol by producing biologically significant amounts of oxalate. This does not happen in man.

In man, as in the the mouse, oral xylitol leads to an increase in glycolic acid production. A little of this glycolic acid is converted to oxalate, but the pathway by which it is formed is readily saturated and the majority of the glycolic acid is excreted as glycolate.

In a recent study on 12 humans, *Dr. Albert Bär* (personal communication) found that the

consumption of 70 to 100 g xylitol per day (i.e. 1 g/kg body weight/day) had no effect on the total urinary excretion of calcium, magnesium, phosphate or oxalate. Also in a study of 916 Hungarian school children (with body weights in the range of 20–30 kg) the daily consumption of 20 g xylitol had no effect on the urinary calcium: creatinine ratio or on the urinary oxalate: creatinine ratio. These negative findings confirm and extend the earlier studies of *Mäkinen et al.* (1981) and *Forster et al.* (1981).

By contrast to the almost complete lack of any effect of xylitol on urinary oxalate excretion in these human studies, the consumption of rhubarb or spinach can increase daily urinary oxalate excretion by as much as 6- or 7-fold in man.

It is therefore safe to conclude that the consumption of xylitol by humans, certainly at levels of up to 1 g/kg/d, carries no risk of increased calcium oxalate bladder stone formation and, therefore, no risk of bladder cancer.

Similarly, it is safe to conclude that the consumption of xylitol poses no risk of adverse effects on the adrenal medulla in humans. In the rat where such effects have been seen, there is persuasive evidence that they are secondary to increased calcium absorption. Humans - both adults and children - exposed to xylitol at dose rates of up to 1 g/kg/day exhibit no enhancement of urinary calcium levels which would be indicative of increased calcium absorption.

In any case the kind of adrenal medullary hyperplasia and neoplasia seen in lactose-treated and polyol-treated rats seemingly do not occur in humans.

Although there are more laboratory data for xylitol than for sorbitol or other polyols, it is likely that the same arguments apply as far as the adrenal medulla is concerned.

Since sorbitol and lactose are not metabolised to glycolic acid, their consumption does not entail any risk of increased oxalate formation. It is not known, however, if their presence in the diet affects the absorption of oxalate. In any case, there is no evidence from studies in rats and mice that either of these substances poses a risk of stone formation or bladder tumour formation even when they are administered at dietary levels as high as 20%.

One final comment is warranted. There are limits to the oral doses of sorbitol or xylitol which humans will tolerate. Above this limit there occurs laxation of the bowels which may be associated with abdominal discomfort. For this reason, there is no likelihood that humans would, in practice, be exposed to the high levels of any of these agents that have been found to cause more serious forms of toxicity in laboratory animals.

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