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SHORTCOMINGS OF CURRENT STRATEGY FOR TOXICITY TESTING OF FOOD CHEMICALS: POLYOLS

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Introduction

Several philosophical misconceptions have interacted to create apparent problems of toxicity in relation to the polyols which in reality were never there.

The first philosophical misconception is that an increased incidence of neoplasia in an animal test, irrespective of the circumstances, indicates that the test substance is a carcinogen. The second is that all carcinogens are equally dangerous and that no level of exposure is safe. The third is that in order to increase the sensitivity of tests for chronic toxicity and carcinogenicity, it is sensible and appropriate to increase the daily exposure dose by one, two or more orders of magnitude above the level of human exposure, provided that the animals can survive the acute and subacute effects of such exposure. The fourth is that neoplasia as an endpoint in carcinogenicity tests is independent of effects on nutritional, hormonal or physiological status.

During the past decade the realization that non-genotoxic mechanisms can be strongly implicated in carcinogenesis has steadily spread. Initially, the term 'tumour promotion' was often used to explain the production of tumours by non-genotoxic agents. However, as documented elsewhere (Roe, 1988a,b), in many forms of non-genotoxic carcinogenesis there is no evidence of prior tumour initiation. In these cases the genetic changes evident in the neoplasms that eventually arise almost certainly occur as a secondary and not a primary event. That is to say, exposure to the non-genotoxic agent in question creates a situation in which mutation is more likely to occur than under normal circumstances. Theoretically, the agent may do this by increasing the rate of metabolism and/or cell turnover in the target tissue and thereby increasing the rate of spontaneous generation of electrophiles, or alternatively by overwhelming cellular mechanisms for repairing DNA damage caused by such endogenous electrophiles. Nevertheless, the use of the term 'tumour promotion' would now seem to be too limiting. The term 'epigenetic' is certainly to be preferred. However, I favour, simply, 'non-genotoxic carcinogenesis'.

It is now widely understood that the guiding principle underlying the setting of the acceptable daily intake (ADI), which has worked reasonably well for food additives and food contaminants, cannot be applied to nutrients and major food ingredients for two reasons. First, in the case of a substance to be used at a concentration of more than 1% in food it is impossible to calculate an ADI for man calculated as one-hundredth of the highest no-observed-effect level in animals. Secondly, disturbances of nutritional, hormonal and physiological status directly and often non-specifically influence the incidence of ageing-related diseases, neoplasia, hormonal status and various non-neoplastic diseases (Conybeare 1980 and 1986; Roe, 1981 and 1988c; Tucker, 1979).

It is against this background that the toxicological evaluation of the polyols needs to be reviewed.

Abbreviations: ADI = acceptable daily intake; FASEB = Federation of American Societies for Experimental Biology.

The polyols in question

Substantial toxicological information exists for xylitol, sorbitol, mannitol and lactitol (Federation of American Societies for Experimental Biology (FASEB) 1979a,b and 1986; Roe, 1989a). The information for other polyols is more fragmentary. A priori one would not expect substances like these to be genotoxic and the tests for genotoxicity that have been carried out have virtually all given unequivocally negative results.

Pathological effects of polyols and those of lactose and other poorly digestible carbohydrates

There is nothing unique about the response of animals to orally administered polyols. All the effects that have been seen in laboratory rodents exposed to high doses are no more than disturbances of physiological status caused by the feeding of diets containing unnaturally high concentrations of poorly digestible carbohydrates. Essentially, the same group of interrelated responses has been seen not only with several different polyols but also with lactose and to a lesser extent with certain chemically modified starches and with polydextrose.

The group of responses consists of: (i) enlargement of the caecum; (ii) laxation; (iii) enhanced calcium absorption from the intestinal tract; (iv) nephrocalcinosis; and, in rats, (v) adrenal medullary hyperplasia and neoplasia.

These effects of polyols and other poorly digestible carbohydrates have long been recognized but they were published in the nutritional literature, which most toxicologists consult rarely, and not in toxicology journals. For this reason toxicologists and regulators were taken aback particularly by the adrenal medullary and renal effects of feeding unphysiologically high concentrations of

polyols and chemically modified starches etc. to rodents when these effects began to be reported some 10 years ago.

Many factors affect calcium absorption from the gut and research on the mechanisms involved is still actively in progress. However, it has been known for over 60 years that dietary carbohydrate facilitates the absorption of calcium. In the case of easily absorbable monosaccharides and of disaccharides and natural starches that can easily be broken down to absorbable monosaccharides, most of the absorption of the carbohydrate, and so also of calcium, takes place in the duodenum and jejunum. But with dietary overloading with carbohydrates that are less readily broken down to absorbable monosaccharides, the length of the gut involved in the absorption both of monosaccharides and of calcium increases. If the breakdown of carbohydrates is largely, or wholly, dependent on enzymes produced by the microflora of the large bowel, as it is in the case with certain chemically modified starches, and with polyols or lactose overload, then a single explanation of all the adverse effects of the feeding of these substances becomes apparent. The caecum enlarges to accommodate its increased metabolic role and because the creation of smaller molecules from larger ones within the lumen of the bowel causes water to flow into the lumen by osmosis. The caecal enlargement and the diarrhoea are thus explained. Next, the breakdown of more complex carbohydrates within the large bowel results in the formation of absorbable carbohydrates in this part of the intestinal tract and calcium is absorbed along with these (including calcium secreted into the lumen of the small bowel). As a consequence, in small rodents, calcium absorption may be increased many-fold.

Species and strains of rodent vary in their ability to cope with increased calcium absorption. Thus, in mice some deposition of calcium may occur in the pelvic region of the kidney, but actual damage to the kidney is not usually seen (Baer, 1985b). In hamsters, high dietary levels of a highly substituted chemically modified starch led, in one study, to acute tubular nephropathy that would have proved fatal had the study not been terminated (Newberne and Buttolph, 1979). Acute tubular nephropathy has also been seen in dogs exposed to dietary polydextrose, which led intermittently to hypercalcaemia (Schach von Wittenau, 1981). But it is in rats that the effects of enhanced calcium absorption are most varied and have been most studied. This species is seemingly less able than others to cope with chronically increased calcium absorption secondary to the prolonged feeding of poorly digestible carbohydrates. Consequently they develop various forms of nephrocalcinosis, including the pelvic form, the cortico-medullary form, acute tubular nephropathy and actual calculus formation (De Groot and Feron, 1976). In addition, they exhibit changes in the adrenal medulla, starting with focal hyperplasia and progressing to the development of benign and malignant tumours (for reviews see Roe, 1989a; Roe and Baer, 1985). Adrenal medullary proliferative disease has been seen not only in rats exposed to several different polyols or to lactose but also in rats exposed to other agents that disturb calcium homoeostasis in the direction of hypercalcaemia (F. J. C. Roe, unpublished data, 1989). Recently, we showed that the incidence of adrenal medullary proliferative disease in untreated control rats varies directly with the severity of chronic progressive nephropathy, a degenerative disease to which overfed rats of many strains are especially prone (F. J. C. Roe unpublished data, 1989). This form of nephropathy compromises the maintenance of calcium homoeostasis with consequent parathyroid hypertrophy, widespread metastatic calcification and increased adrenal medullary proliferative disease.

An extra question in the case of xylitol

Except in one aspect the mechanisms underlying the unphysiological and pathological effects of administering high dietary concentrations of lactose, sorbitol, mannitol, lactitol or xylitol to laboratory rats and mice are similar. The exception is that xylitol is metabolized by way of glycolate to oxalate.

In mice fed on diets containing 10 or 20% xylitol this pathway can lead to sufficient oxalate production to increase the risk of calculus formation in the renal pelvis and bladder. Increased oxalate formation is peculiar to xylitol among the polyols that have been studied.

Relevance for man

The relevance for man of the effects in rats and mice exposed to excessive dietary concentrations of lactose, polyols or other carbohydrates has been discussed (Roe, 1989a) and the following main conclusions reached:

(i) Caecal enlargement. The caecal enlargement associated with the feeding of high dietary concentrations of lactose or polyols to rodents has not been seen in humans. This probably relates to the fact that rats eat per day up to 25% of their own body weight (e.g. equivalent to a human eating 14–18 kg food per day). It is conceivable that a human who ate this amount of a diet containing 20% lactose would develop caecal enlargement! However, this cannot be tested so that the closest equivalent of caecal enlargement that occurs in man is the flatulence, abdominal discomfort and mild laxation observed in lactase-deficient individuals when they consume lactose and in response to suddenly increased exposure to a polyol (e.g. over-indulgence in a fruit containing sorbitol). If daily consumption of polyols is increased gradually, adaptation occurs. In these circumstances flatulence, abdominal discomfort and laxation do not constitute a problem.

(ii) Hypercalciuria and calculus formation. There is no evidence that the consumption of lactose or any of the polyols (including xylitol) materially disturbs calcium metabolism and thereby increases the risk of renal-tract stone formation in humans (Baer, 1985; Baer and Osterhelt, 1985; Cochet *et al.*, 1983; Francis *et al.*, 1986).

(iii) Adrenal medullary proliferative changes. The marked enhancement of calcium absorption seen in rats in response to high dietary intakes of lactose, polyols or other poorly digestible carbohydrates does not occur in man. Furthermore, the clear association between enhanced calcium absorption and other con-

ditions leading to hypercalcaemia seen in rats appears to be a species-specific phenomenon. In mice enhanced calcium absorption is not associated with evidence of adrenal medullary proliferative disease. In man no evidence of increased risk of adrenal disease has been reported in association with hypercalcaemic states. This topic was reviewed by Roe (1989a) and Roe and Baer (1985).

Effects on the testis

In rats in one study (Sinkeldam *et al.*, 1983) exposure to high dietary levels of lactose led to an increased incidence of Leydig-cell tumours of the testis. This effect has not been reported for any of the polyols. If real, it at present lacks explanation. It is well known that some strains of laboratory rat are peculiarly prone to developing this type of testicular tumour spontaneously. No other species is similarly susceptible. It is reasonable to conclude, as did FASEB (1986), that man is not at risk from this apparent effect of lactose in rats.

Conclusions

The calculation of ADIs for man based on the application of large safety factors to findings in animal studies is inappropriate for major nutrients such as polyols or lactose. Attempts to do this combined with a failure to distinguish between genotoxic and non-genotoxic mechanisms in carcinogenesis led to confusion and disarray among toxicologists and food safety regulators. Instead, ADIs need to be based on a common-sense interpretation of available human and animal data that takes into account the implications of incorporating macronutrients into diets on nutritional, hormonal and general physiological status. On such a common-sense basis, 'safety assurances' may be provided by data from studies in animals where there is only a small margin (e.g. two-fold) between the level that causes adverse effects in animals and a level of safe use for man.

A first requirement in the safety evaluation of nutrients and major food ingredients should be to study their absorption and metabolism in different species, including man. Given this information it may be deemed unnecessary to go through the full gamut of tests normally required for a xenobiotic food additive or contaminant. If further tests are required, be they short term or long term, the first priority must be to see whether at ordinary levels of inclusion in the diet there are disturbances of physiological or hormonal status or of mineral balance. Also, it must be borne in mind that apparent effects of exposure might be due, not to the substance under test, but to the reduced consumption of one or more other dietary constituents that it replaces. In the case particularly of long-term tests, one needs to be aware of the major effects that differences in caloric intake, dietary composition and mineral balance may have on longevity, and on the incidence of many non-neoplastic, neoplastic and ageing-associated diseases (Roe, 1989b).

The fact that most long-term rodent studies are conducted under conditions of over-nutrition, which lead to high incidences of physiological and hormonal disturbance in untreated control groups, unnecessarily compromises the toxicological evaluation of macronutrients such as lactose and some of the polyols.

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