TNO-CIVO Workshop on Low Digestibility Carbohydrates : Biological, industrial and regulatory aspects, November 27-28, 1986. Contributions by Dr. Francis J.C. Roe to:

FORUM DISCUSSION:
IS THERE A COMMON MECHANISM UNDERLYING THE EFFECTS OBSERVED WITH LOW DIGESTIBILITY CARBOHYDRATES IN ANIMAL STUDIES AND HOW RELEVANT ARE THESE EFFECTS FOR MAN

I-105 Roe : I think there are two different situations: In the first, caecal enlargement (CE) is associated with the presence in the lumen of the caecum of substances which are going to be converted into absorbable monosaccharides (MS). In this case, Ca absorption is likely to be increased because calcium rides through the gut mucosa on the back of monosaccharides (Vaughan and Filar, 1960; Bergeim, 1926). In the other situation, CE is not associated with the presence in its lumen of substances which can be broken down to MS, and no increase in Ca absorption is to be expected. Certainly CE is not necessarily due to bacterial overgrowth, since it occurs in germ-free animals. It is frequently suggested that CE is an osmotic effect secondary to the fact that when large molecules are broken down to small molecules they attract more water.

During recent years, there has been an explosion of knowledge in peptide hormones which control many of the functions of the gut. It distresses me that, as toxicologists, we are failing quite miserably in that we are not studying the possible involvement of disturbance of peptide hormone status in relation to CE in the rat.

I-203 Roe : Are we not getting our words mixed up? What do we mean by diarrhoea? We know what it is in humans, but are we all defining it in the same way when it comes to the rat? Are we talking about consistency of stool, amount of stool, frequency of stool or staining around the tail? In practice, the term diarrhoea is applied quite loosely, if I may use that word! We should be absolutely specific what we mean by diarrhoea in a rat.

I-528 & I-622 Roe : I agree with Herb Blumenthal that we are involved in an enormously complex area involving numerous inter-related mechanisms and numerous variables. A serious problem is that, although a huge number of experiments have been carried out and reported in the literature - a lot of them in the older 'nutrition' literature - nobody has been able to control all the important variables in the same experiment.

For present purposes, I suggest that, before we consider mechanisms, we need to recognise that several different disturbance patterns are discernible in rats. Immediately I can think of four such distinct patterns: the first is one associated with increased calcium absorption, the second is one associated with overnutrition, the third is one associated with opportunity or lack of opportunity for sexual indulgence, and the fourth with dietary levels of minerals, particularly Ca, Mg and P.

The pattern of effects associated with increased calcium absorption includes caecal distension, increased urinary calcium, pelvic nephrocalcinosis, pelvic epithelial hyperplasia, increased adrenal medullary proliferative disease and, possibly, increased incidence of Leydig-cell tumours (Roe and Baer, 1985; Hodgkinson et al, 1982).

The pattern of effects associated with overnutrition includes premature death, increased incidence and severity of chronic progressive nephropathy, increased cortico-medullary nephrocalcinosis, increased adrenal medullary proliferative disease, increased pituitary and mammary tumours, increased serum prolactin, irregularity of oestrous cycling and premature loss of fecundity, increased incidences of other endocrine tumours including pancreatic islet-cell tumours and C-cell

tumours of the thyroid gland (Conybeare, 1986; Harleman et al, 1984).

The patterns of effects associated with enforced celibacy or with intermittent sexual overindulgence are presently less clear cut and require further research. Litter-bearing protects females to some extent from pituitary tumour development. In males the most amusing information I have is as follows:-

In an experiment, nicknamed "George's experiment" for reasons that it is not necessary for me to dilate upon, two groups of male Wistar rats were maintained singly-housed all the time (Group 0) or singly-housed but provided with a fresh virgin for one week, every alternate week (Group G). After two years, much to George's chagrin, the incidence of LCT was higher ($p<0.01$) in Group G than in Group 0! [According to George, it was the repeated frustration of the intervening weeks that caused the trouble!] (These findings with others of possibly less interest will be submitted for publication during 1987: lots are presently being drawn for authorship).

Finally, there are the patterns relating to variation in dietary, mineral or Vitamin D status. Diets exhibiting phosphorus excess or deficiency, a ratio of calcium:phosphorus less than unity, or magnesium deficiency can all give rise to one or more form of nephrocalcinosis. Vitamin D deficiency leads to hypocalcaemia and adrenal medullary hypofunctioning. Restoration of the blood calcium to normal is associated with restoration of adrenal functioning to normal (Brion and Dupuis, 1980).

The recognition of these different patterns have been often blurred by investigators referring to conditions such as

progressive nephropathy, pelvic nephrocalcinosis, adrenal medullary proliferative disease, and pituitary, mammary and testicular tumours as 'age-associated'. Of course, they are age-associated, everything bad is age-associated! But just to label something as age-associated does not establish that the main mechanism involved is an ageing process. Overnutrition is a principle cause of all the conditions I have mentioned.

In a recent study, I observed in male rats dose-related incidences and severities of (i) chronic progressive nephropathy, (ii) metastatic calcification and adrenal medullary proliferative disease. When we looked at the results more closely, we found that if we standardized for nephropathy grade, treatment was without effect on the adrenal medulla.

II-016 Roe : In most laboratories - perhaps less so in CIVO than in many other laboratories - ad libitum-fed rats become quite huge, weighing over 1 kg. However, one should not confuse overfeeding with obesity. The two are obviously related, but obesity is not always a good marker for the other adverse effects of overfeeding.

Overfed rats show multiple endocrine disturbances, the precise spectrum varying from study to study. By restricting the food intake, animals may be protected from these endocrine disturbances. The most convenient way to restrict feeding is to limit the period of access to food to 5-6 h/day. Feed-restricted animals don't die prematurely and get fewer pituitary tumours, mammary tumours, and pancreatic islet-cell tumours.

II-042 Roe : I would expect fewer LCT, less PN and little or no adrenal medullary proliferative disease. That would be my expectation.

II-084 Roe : Rats fed ad libitum nibble food all through the night and eat nothing during the day. Rats given access to food for only 6 hours/day start off with an extremely big meal as soon as food is provided. And then, at the end of the period of access, perhaps because they hear someone coming down the corridor to remove the food baskets, they have another big meal. Some rats additionally have a third meal half-way between the other two. Thus, the provision of food during just 6 hours per day converts a night nibbler into a daytime meal-feeder (Conybeare, 1986).

II-106 Roe : I agree with that!

II-112 Roe : One should be very careful in the use of the word 'sensitive'. In my opinion one should only ever compare animals which are in a physiological status with humans which are in a physiological status.

II-118 Roe : Well, by comparison with the overfed rat, we humans are paragons of normality! Who knows of even a sub-group of the human population wherein virtually 100% of the women develop pituitary and multiple mammary tumours and or almost all the men develop LCT, half of them develop adrenal medullary tumours and many of them develop endocrine and/or exocrine tumours of the pancreas?

II-181 Roe (addressing DeGroot) : In your talk on isomalt, you presented a table summarising all your results. It seemed to me that two different patterns of effect were discernible. First, there were beneficial effects secondary to the avoidance of overnutrition in the top-dose group. Here you saw reduced weight gain, reduced cortico-medullary nephrocalcinosis, and better survival. Secondly, however, the same animals exhibited increased pelvic nephrocalcinosis. This was, no doubt, secondary to the effect of the test material in increasing calcium absorption from the gut and urinary calcium excretion.

It is not my view, therefore, that none of the reported effects of polyols would have been seen if the experiment had been conducted under conditions of diet restriction. All I am saying is that at least two different patterns of response have become interwoven - one relating to effects on calcium absorption and one relating to those of overnutrition.

II-204 Roe : Yes! Pelvic nephrocalcinosis is a consequence of increased calcium absorption. As I say, you have two effects going on in your study: increased calcium absorption which is driving the adrenal medullary tumours up and causing the pelvic nephrocalcinosis and, at the same time, beneficial effects secondary to reduced food intake including better survival and reduced cortico-medullary nephrocalcinosis.

Now there is one other thing that needs to be pointed out. In your isomalt study, the maximum dietary level tested was 10%. This needs to be distinguished from the 20% level which gave rise to a spectrum of adverse effects in the cases of lactose, xylitol and sorbitol. I suspect that a diet containing 20% isomalt would have given rise to the same spectrum of effects as 20% lactose.

II-224 Roe : No! It is not completely different. They are two patterns of response with overlapping spectra of causative factors. We know, for instance, as a result of work carried out at CIVO that if dietary lactose levels are pushed up high enough rats develop cortico-medullary nephrocalcinosis as well as PN. On the other hand, the corticomedullary nephrocalcinosis, which is very commonly seen, or used to be very commonly seen, in female rats is partly an overfeeding related phenomenon and partly a consequence of dietary mineral imbalance in the form of Mg deficiency. Different spectra of mechanisms underly pelvic nephrocalcinosis and cortico-medullary nephrocalcinosis, but the spectra overlap.

II-284 Roe : I have a completely open mind. I don't think we presently have much of a clue as to what causes LCT in rats, although I suspect that overnutrition plays a role. All we know is that LCT occur in undescended testicles and arise in rats in the wake of testicular atrophy due to acute cadmium poisoning. When seminiferous tubules are destroyed, first castration cells appear in the pituitary and then LCT arise in the testis. How this relates to what happened in the lactose and lactitol studies I really don't know!

II-318 Roe : It would be interesting to look at the circulating hormonal status of rats before and after they develop Leydig-cell tumours and to compare this with their fertility. I wonder whether castration cells appear in the pituitary before F344 rats develop Leydig-cell tumours?

II-431 Roe : If I were a purveyor of polyols, I would be inclined, in the first instance, to confine any further studies of mechanisms involved in Leydig cell tumorigenesis to lactose on the grounds that lactose produces the effect and is not really vulnerable to regulatory proscription. I would give priority to comparing the effects of diets containing 10 or 20% lactose (a) under conditions of ad libitum feeding and (b) under conditions of 6-hour/day limited access to food. In such experiments, I would very carefully measure the mineral contents of the diet, especially with respect to Ca, Mg and P. In subsequent studies, I would want to see to what extent the apparent effects of lactose on the kidney, adrenal and testis are influenced by changes in the mineral content of the diet and by other changes in dietary composition (e.g. protein content). In all these studies, I would want to have adequate information with regard to circulating hormone levels (e.g. prolactin, growth hormone, insulin, etc.) and urinary excretion of catecholamine metabolites. Also, I would pay detailed attention to all aspects of the endocrine system at necropsy and during the histopathological evaluation of effects on tissues. It would not really surprise me if such research enabled one to define conditions in which a diet containing 20% lactose may be given to rats over their life-time without its causing increased incidences of testicular or adrenal medullary tumours.

II-463 Roe : In many laboratories mice under experiment are also overfed. However, the adverse effects of the overfeeding of mice are rather different to those of overfeeding rats. Increased incidences of liver, lung and lymphoreticular neoplasms, along with obesity and reduced longevity, are the main effects. Disturbance of the endocrine system is a less obvious consequence of overfeeding in mice than in rats.

II-648 Roe : In humans conflicting findings have been reported in relation to the effects of lactose on calcium absorption (Cochet et al, 1983; Dupuis & Fournier, 1963; Greenwald et al, 1963; Kocian et al, 1973; Ziegler & Fomen, 1983; Pansu et al, 1971). The picture seems to be that in individuals whose gastrointestinal tract secretes the enzyme lactase,and in whom lactose is broken down to glucose and galactose and the absorption of these monosaccharides may be associated with some enhancement of calcium absorption. However, in lactase deficient individuals dietary lactose does not enhance calcium absorption, indeed it may even have the opposite effect. Races differ in lactase competence and it is common among all races for the incidence of lactase deficiency to increase with age.

I was recently involved in a study which was going to make me a millionaire. Since sorbitol increases calcium absorption in rats, I thought that this polyol might protect post-menopausal ladies from the osteoporosis which predisposes them to fracturing their femora. Borrowing the name of the wartime children's dietary vitamin-C supplement, i.e. "Rose Hip Syrup", I proposed the name 'Roe's Hip Syrup' for the proposed new potion that was destined to put hundreds of orthopaedic surgeons into dole queues. Alas, it all went wrong! Francis et al (1986) found that sorbitol, rather like lactose in lactase-deficient individuals, far from increasing calcium absorption in post-menopausal women, if anything, actually reduced it.

As to the relationship between excessive calcium absorption and adrenal medullary proliferative disease, Professor Oliver Wrong at University College Hospital in London (personal communication) looked through the records of some 600

individuals with hypercalcaelmia associated with hyper-parathyroidism. In only one of these was there also a phaeochromocytoma and this was suspected of being a case of the multiendocrine disorder, Sipple's disease.

Leydig-cell neoplasia is rare in man although, as in rats, marked hyperplasia of these cells is a common feature of the incompletely distended testis (Willis, 1960). I sometimes wonder whether the lesions which are usually reported to be Leydig-cell tumours in rats are not, in most cases, examples of florid hyperplasia rather than of neoplasia. Certainly there is no clear boundary line between hyperplasia and neoplasia in the case of Leydig-cell lesions in the rat testis.

III-53 Roe : In the mouse study on xylitol and sorbitol conducted at the Huntingdon Research Centre (Hunter et al, 1978), sucrose was used as a control. We should not forget that there was a significant excess of liver tumours in the sucrose 'control' group.

III-172 Roe : I would like to make a final statement. In all the studies I have been involved in, lactose has been the most potent of the agents looked at. Whatever a polyol can do, lactose can do it as well or better, with the exception of the bladder tumours in mice with xylitol. So, in any strategic approach, my first priority would be to see what effect would lactose have in physiologically normal animals maintained under conditions of diet restriction.

Secondly, I should want to define the effects of 'physiological' levels of lactose in the diet in physiologically 'normal' animals. One cannot extrapolate to

man from feeding studies in which rodents are fed on diets containing 10 or 20% lactose.

Thirdly, in relation to elucidating the mechanism whereby high dietary concentrations of lactose or polyols cause adrenal medullary proliferative disease in rats, the first priority must be to look for early effects and to compare these in (a) hormonally abnormal overfed rats and (b) physiologically normal diet-restricted rats. In such studies it would be important to attach particular attention to catecholamine and prolactin status as well as to all aspects of calcium homeostasis.

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