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ANIMAL MODELS – A CRITICAL EVALUATION

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This review addresses the need to evaluate the biological effects of novel foods, how this can be achieved and the role of animal models in this process. The selection of an appropriate model or combination of approaches is most important, together with an awareness of the shortcomings of that particular method. In mammals the gastrointestinal tract not only acts as the site of absorption of nutrients and non-nutrients, but provides a protective barrier between the environment and the body. As such this interface is exposed to relatively high concentrations of the individual chemicals that are dietary components. It is therefore essential to understand how novel foods can change the structure, growth and function of the gut in particular. Effects may be both beneficial and detrimental and have an impact on the function of other organs in the body. However, due to the wide-ranging implications of this broad topic, the discussion here will focus on events within the gastrointestinal tract, in the context of the evaluation of novel foods.

INTRODUCTION

The mucosal epithelium lining the mammalian gastrointestinal (GI) tract is one of the most dynamic tissues in the human body, with around 10^7 cells per hour being replaced in the human colon. It provides a protective barrier between the intestinal lumen and the internal organs, whilst facilitating the processing of dietary components and allowing the passage of nutrients and other small molecular species into the body. Its functional and anatomical stability is maintained by rapid cell replacement, driven by luminal nutrition. In the small bowel, the digesta provide the cells of the brush border with a source of energy for cell proliferation and metabolism, whereas in the colon the major fuel is short chain fatty acids produced by microbial fermentation in the gut lumen. The diet thus plays an essential role in maintaining functional stability and an optimal microflora.

Novel foods, especially those with increased functionality in this respect are under constant development, and are frequently the result of the commercial exploitation of fundamental research observations. For example products may contain new components or mixtures which modify the delivery of nutrients or provide a source of micronutrients or bioactive non-nutrient compounds. They may also consist of altered matrices such as emulsions, or be incorporated into specialised delivery systems such as encapsulation [Cheng *et al.*, 1994] or micro-encapsulation to target a specific region of the GI tract. The majority of novel foods are specifically designed to be beneficial to human health, or at least be without detrimental effect. Thus potential benefits need to be assessed at the whole body, the organ and the cellular or tissue levels and any risks evaluated, particularly with respect to the more subtle physiological changes that they invoke.

It has been appreciated for some time that there are strong associations between diet and health. Dietary changes can profoundly affect the lining of the GI tract which in turn can influence general well-being, and enterocyte and colonocyte integrity and function are therefore primary targets for intervention. Intestinal cells are exposed to a wide variety of dietary chemicals at relatively high concentrations, in an environment that is often harsh, and are therefore very susceptible to disease. Novel foods are frequently marketed as sources of a range of biologically active materials, having potentially protective effects against the onset of disease, or with therapeutic activity, both in the long and short term. For example, the absorption and digestion of macronutrients is reduced when the viscosity of the small intestinal contents is increased in the presence of soluble non-starch polysaccharides such as guar gum [Johnson & Gee, 1981; Gee *et al*., 1983]. Similarly binding between for example saponins and bile salts reduces cholesterol bioavailability [Francis *et al*., 2002; Gee & Johnson, 1988; Oakenfull & Sidhu, 1990]. Both of these effects are of potential benefit to Type II diabetics and clinically obese individuals. However, it should also be borne in mind that compounds such as saponins can compromise cell permeability and thus negate the barrier effect of the mucosal brush border. This has implications regarding the uptake of molecules normally excluded from the body and the subsequent development of allergies. Ingestion of fermentable carbohydrate can also change the pattern of secretion of gut hormones, particularly those associated with gastric emptying and gut motility [Gee *et al.*, 1997a], and viscous non starch polysaccharide (NSP) raises

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rates of cell proliferation in the small and large bowel. From the point of view of colonic polyp development, strategies that lower cell birth (mitosis) or increase cell suicide (apoptosis) are deemed to be protective against the development of bowel cancer.

THE CHOICE OF A MODEL

Evaluation of the effects of novel foods on the GI tract is therefore essential in an assessment of their safety and efficacy. Methodologies range from the culture of human cell lines to animal studies *in vitro* and *in vivo* and human intervention trials. Although none is a perfectly comprehensive model system each has a role to play, and a combination of two or more techniques is often the best approach. Cell culture is a rapid *in vitro* technique ideally suited to screening studies, and lines such as CACO2, that spontaneously transform themselves from colon cancer cells into the phenotype of enterocytes at maturity, provide a useful model to assess small intestinal absorption. The growth of tissue explants from biopsies or surgical waste *in vitro*, in which tissue structure is maintained, can provide further information, but it must be remembered that cells under these conditions are still somewhat isolated and constrained.

At the other end of the scale, human intervention trials are obviously the closest model to the free-living human being, but often participation in this type of study can modify behaviour and routine sampling is restricted to collection of blood, urine and faeces, although biopsies and surgical waste are sometimes available. Such studies are also costly, lengthy and complex to organise and carry out, requiring exhaustive prior ethical approval to comply with current legislation. The use of primates for research is highly restricted on both ethical and financial grounds, and consequently small mammals have been employed extensively as animal models, providing a wide variety of *in vitro* and *in vivo* techniques with the possibility of virtually unlimited sampling. Frequently used animals are the mouse, rat, guinea pig, hamster and rabbit, however it is essential to be aware of subtle metabolic differences between humans and available animal species before making a choice. For instance there are variations in lipid metabolism between the laboratory rat and man, but the hamster, having a gall bladder, is a better model for some aspects of small bowel metabolism. The pig is considered to be one of the most appropriate models for the human gut, but in terms of size, cost and handling may prove to be impractical in some situations.

Thus the role of animal models in the evaluation of novel foods has an important niche. However, the choice of animal must be made with care, bearing in mind the appropriateness and limitations of the model, the availability and cost of animals and ethical and licencing considerations. Experiments should be designed carefully in order to maximise the information gained and obtain sufficient data for statistical analysis. On a more practical note, the size of individual organs has to be sufficient to permit the procedures planned, and for this reason rats are frequently used to evaluate the biological effects of new food components and novel foods. In the paragraphs that follow although most of the examples cited use the rat, the techniques described can be applied to most of the animal species mentioned above.

IN VIVO STUDIES TO ASSESS EFFECTS ON GROWTH, DISTRIBUTION AND FATE

Basic *in vivo* animal studies usually take the form of feeding experiments to assess the effects of a "test" material on food and water intake and to measure growth and food conversion ratios [Johnson *et al.*, 1989, 1990]. A variety of techniques is available to measure transit time, and the effects that a novel food may have on the flow of digesta through the intestine [Carson & Smith, 1983; Akiyama *et al.*, 1995]. Information on bioavailability or metabolic responses can be obtained from sequential blood sampling, for example from a tail vein, and effects on gut bacteria can be extrapolated from faecal analysis. Following the death of the animal, parameters such as organ mass, GI tract length, viscosity of intestinal contents and caecal pH can all be recorded. For a more detailed assessment of cellular changes, tissue samples can be fixed for microscopy or extracted for analysis. The distribution and fate of a particular dietary component can be tracked either chemically or using radioactive tracers. To evaluate the protective effects of a particular novel food, biomarkers of health or disease can be determined in, for example, intestinal mucosal scrapes, blood or target organs, to establish efficacy. Alternatively the use of an animal model predisposed to a particular disease or presenting with symptoms, facilitates investigation of protective and/or therapeutic activity. Genetically modified mice, such as the *min* mouse which is nul for the *apc* gene and develops numerous colonic polyps, are being bred to model a variety of disease states. These so called "mouse knock-out animal models" have been available for some time, but very recently the first knock-out rat strain has been bred, thus broadening the scope of this technique.

MODELS OF ABSORPTION AND METABOLISM

In order to probe the mechanisms of absorption *in vivo*, segments of gut can be isolated in an anaesthetised animal and the appearance of the parent compound(s) or metabolite(s) monitored in portal blood. This technique allows the separation of intestinal and hepatic processing, giving further insight into the metabolism of the component(s) under investigation. It can also be used to assess intestinal permeability to macromolecules [Gee *et al.*, 1997b]. A variety of more complex surgical models have been available for some years, mainly using pigs, to permit access to various segments of the GI tract [Pekas, 1965; Noakes & Cranwell, 1977; Lewin *et al.*, 1981], and more sophisticated animal models are currently being developed to mimic the development of inflammatory states by surgical intervention. To probe mechanisms further, preparations of everted rings [Johnson *et al.*, 1986], everted sacs [Johnson *et al.*, 1986], intestinal sheets [Barnett & Licko, 1977], or cannulated everted sacs can be used *in vitro* to study absorption or to investigate effects on the integrity of the mucosal barrier [Gee *et al.*, 1989]. Rinsed intestine is everted and either cut into rings or made into sacs containing buffer, these can then be incubated under

oxygenated conditions in a shaking water bath to determine tissue uptake and transport. Water transport can also be measured under similar incubation conditions using a torsion balance. A variation of the cannulated everted sac technique also offers the possibility of measuring transport at the nanomolar level, where the possibility of contamination from mucosal bathing solutions must be virtually eliminated. In this case the tissue is ligatured to a syringe, providing access to the internal compartment and removal of the serosal solution for analysis.

EFFECTS AT THE CELLULAR LEVEL IN THE INTESTINE

The biological effects of novel foods can also be evaluated at the cellular level by assessing changes in appropriate biomarkers. Diet can have a profound influence on a wide variety of cellular functions, for example the secretion of digestive enzymes [Johnson *et al.*, 1984], the activity of transporters [Zhang *et al.*, 1998] and expression of receptors [Grimes *et al.*, 1992; Bamba *et al*., 1993]. It also has the ability to raise or lower rates of cell proliferation in the lining of the intestine. This tissue has the highest turnover in the body, with cells being replaced on average about every two to three days. Stem cells at or near the base of the intestinal crypts are the source of new cells, which then divide several times as they travel up the crypt axis and then differentiate. In the small intestine the process of maturation extends to the overlying villi (Figure 1), and cells are eventually shed from the villus tips. Cell proliferation is a finely controlled balance between mitosis and apotosis. When mitosis predominates tissue hyperplasia occurs, and there is an increased risk of the proliferation of mutated cells and ensuing cancer. It is possible to stain and microdissect fixed intestinal samples and prepare intact crypt mounts [Goodlad *et al.*, 1991] (Figure 2a), which can then be assessed for the incidence of mitosis and apoptosis without the need for sectioning (Figures 2b and 2c). Actual rates of cell proliferation can be measured by the metaphase arrest technique [Johnson *et al.*, 1988] in combination with the whole crypt mount.

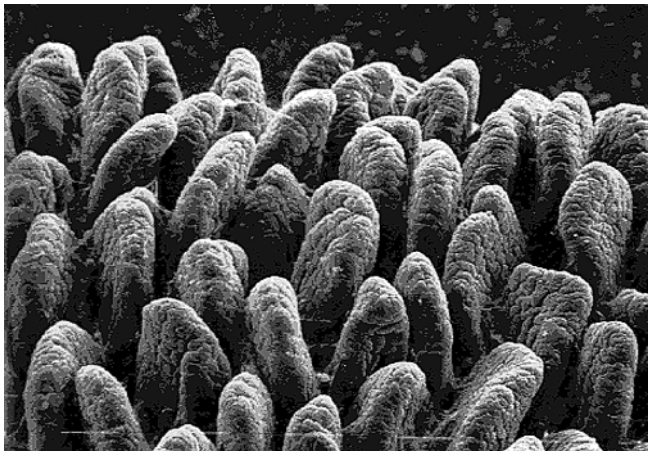

FIGURE 1. Scanning electron micrograph of rat small intestinal villi (x160).

In general, it is accepted that dietary factors that either reduce mitosis or increase apoptosis are potentially protective against the development of bowel cancer. Neoplasia can be induced experimentally by administration of a carcinogen to animals. Aberrant crypts, characterised by gross hyperplasia at the crypt mouth, can then be counted in colonic specimens after 6 weeks [Smith *et al.*, 1998; Gee *et al*., 2000], or tumours enumerated after several months [Caderni *et al*., 2000]. The counting of total aberrant crypt foci (ACF) has been described by some as unreliable, as not all ACF progress to tumours [Wargovich *et al*., 1996]. However this criticism has been recently addressed by Caderni *et al.* [2003] who reported a good correlation between mucin-depleted foci (MDF) and tumourigenesis. Protective effects of novel foods can be investigated by dietary intervention before, during or after treatment with the carcinogen to assess either the "blocking" of the action of induction or "suppression" of development of tumours [Gee & Johnson, 2001]. With increasing information on the rat genome, and the completion of the human genome project, the new discipline of nutritional genomics opens the door to a deeper understanding of dietary interactions with the mammalian body. The use of DNA microarray technology to assess changes at the genome level, and proteomics to monitor post translational responses to dietary change, will potentially enable researchers of the future to design whole diets and perhaps novel foods tailored to particular genetic polymorphisms.

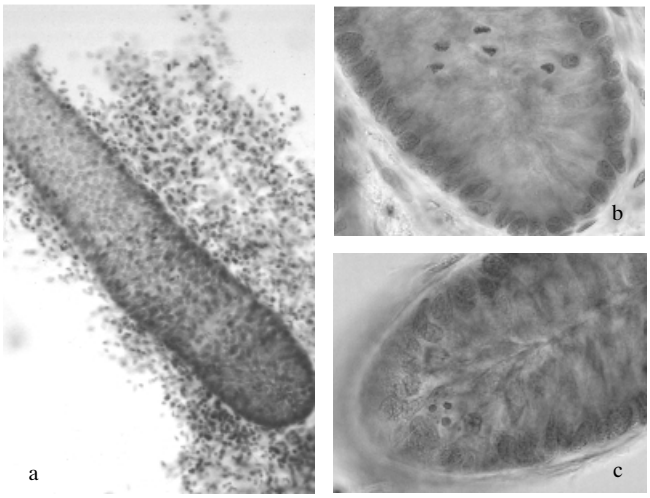

FIGURE 2. Whole stained, microdissected crypt (a), showing cells undergoing mitosis near the crypt base (b) and apoptosis at the crypt base (c).

CONCLUSIONS

Whatever the experimental rationale, the principle of the three R's (*reduction, refinement* and *replacement*) is central to the licencing of the use of animals to model human physiology and biochemistry. Numbers used should be kept to the minimum, whilst being sufficient to provide adequate data for meaningful statistical analysis. Protocols and procedures should be refined to ensure the optimum experimental design, and where possible alternatives such as cell culture should be considered. However animal models can be effectively used to evaluate both the positive and negative biological effects of consumption of novel foods. A broad range of *in vitro* and *in vivo* methodologies is available, but an awareness of their limitations is essential. The various techniques should be used if possible to compliment cell culture and human studies to provide an integrated assessment of potential benefits to

human health. When used with care and in accordance with legislation and ethical considerations, they can provide much useful information which could not be obtained otherwise.

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