CORRESPONDENCE

GM food debate

Sir—Stanley Ewen and Arpad Pusztai (Oct 16, p 1353)[1] raise intriguing questions about the potential of *Galanthus nivalis* agglutinin (GNA) to cause morphological alterations in the intestinal tract and suggest that the lectin's effects may be exacerbated in genetically modified potatoes. However, the ways in which Ewen and Pusztai assess the enteropathic properties of GNA mean that their findings must be interpreted with extreme caution.

The indices of crypt length and jejunal intraepithelial lymphocyte (IEL) count have been used to study intestinal immunopathology for 20 years or more[2,3] but several aspects of Ewen and Pusztai's methodology warrant attention. First, the study relies entirely on image analysis of formalin-fixed, paraffin-embedded sections, which are notoriously subject to shrinkage, distortion, and other fixation artifacts. These problems can be partly overcome by careful choice of well-oriented villus-crypt units combined with exhaustive measurement techniques, but Ewen and Pusztai do not indicate whether they did this. Errors created by measuring crypts in different planes of section on the sample could account for the high variation reported. The fact that the crypt lengths reported (60–90 μm in the jejunum) are much smaller than those normally found in the rat[4] reinforces the view that the measurements may not have been accurate. More sensitive methods for processing and measuring intestinal tissues include non-formalin-based fixatives, microdissection, and direct morphometry of crypt and villus lengths.[2–4] The enterocyte mitotic rate is probably the most sensitive index of intestinal pathology,[2] and this can be measured easily by metaphase arrest or incorporation of bromodeoxyuridine.

Ewen and Pusztai use IEL counts to support their hypothesis that genetically modified potatoes cause jejunal lesions. They state that "IEL are known to increase when non-specific intestinal damage occurs", but an increase in IEL count is specifically a feature of enteropathies associated with activated T lymphocytes.[3] Thus an increased IEL count in animals receiving lectins could be compelling evidence for these materials inducing immunologically mediated damage to the gut. However, Ewen and Pusztai have not shown this conclusively. They do not seem to have counted IELs by a well-established method in which IEL are counted per 100 enterocyte nuclei or as an absolute number per length, volume or area of mucosa. That the technique they used is not ideal is underlined by the numbers of IEL they report, which are in the region of 7–11 per 48 villi. To reconcile these estimates, given that a single column of villus enterocytes in the rat jejunum contains 200–300 enterocytes and the density of IEL in the normal small intestine is 10–20 per 100 epithelial cells, is difficult. The low protein content of some of the diets, referred to by other commentators as a possible source of error,[5] could account for some deviation of IEL numbers from normal but not for such a gross change. However, it is feasible that such a diet might have made the rats more susceptible to the intestinal infections known to cause the kind of changes in IEL and crypts[3] noted here.

The speculation that the lectin caused jejunal crypt hyperplasia via a direct stimulatory effect on crypt cells cannot be substantiated by the data. Hyperplasia implies increased mitotic activity, which was not measured. Also, the time course for these changes is not described, and no parameters of villus pathology are provided. In the absence of this information, it is impossible to say whether the changes in crypt morphology are primary effects of the lectin or secondary to villus damage.

Interactions between lectins, intestinal epithelial cells, and the local immune apparatus is an important and poorly understood area. Appropriate methods for studying the enteropathic effects of lectins are available and are comparatively simple and inexpensive. Application of these techniques may help elucidate the issues raised by this provocative study.

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1 Ewen SWB, Puszati A. Effects of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet* 1999; **354:** 1353.

2 Mowat AM, Ferguson A. Intraepithelial lymphocyte count and crypt hyperplasia measure the mucosal component of the graft-versus-host reaction in mouse small intestine. *Gastroenterology* 1982; **83:** 417–23.

3 Ferguson A. Models of immunologically-driven small intestinal damage. In: MarshMN, ed. Immunopathology of the small intestine. Chichester: Wiley, 1987: 225–52.

4 Clarke RM. Mucosal architecture and epithelial cell production rate in the small intestine of the albino rat. *J Anat* 1970; **107:** 519–29.

5 Kuiper HA, Noteborn HPJM, Peijneburg AACM. Adequacy of methods for testing the safety of genetically modified foods. *Lancet* 1999; **354:** 1354.

Sir—While much of the debate has focused on the nature of the diets studied by Stanley Ewen and Arpad Pusztai[1] and possible differences between them, one central question is the effects of these on cell proliferation in the gut. Ewen and Pusztai talk about "proliferative effects" when they have not measured intestinal cell proliferation but merely crypt depth. Crypt depth might reflect hypoplasia and hyperplasia but this has yet to be shown. Various methods can be used to measure intestinal epithelial cell proliferation, such as the numbers of dividing cells in optimally sectioned crypts, but for definitive conclusions we need measurements related to the rate of crypt or gland cell production;[2] the size of the epithelial population also needs to be assessed appropriately. Perhaps the best way of doing this is to use metaphase arrest and the micro-dissection method,[3] in which not only the rate of crypt cell production but also good measurements of crypt and villus size can be captured simultaneously.

Another point is that many such studies can be confounded by concomitant changes in the denominator,[4,5] and the data on intraepithelial lymphocytes, with sectioned villus as the denominator, could be subject to the same criticism.

We hope these comments will help to ensure that if these studies are repeated (as they should be), robust, rapid, and reliable methods for assessment of cell proliferation are used.

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1 Ewen SWB, Pusztai A. Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet* 1999; **354:** 1353–54.

2 Wright NA, Alison MR. The biology of epithelial cell populations. Oxford: Oxford University Press, 1984.
3 Goodlad RA. Microdissection-based techniques for the determination of cell proliferation in gastrointestinal epithelium: application to animal and human studies. In: Celis JE, ed. Cell biology: a laboratory handbook. San Diego and London: Academic Press, 1994; 205–16.
4 Goodlad RA. The whole crypt and nothing but the crypt. *Eur J Gastroenterol Hepatol* 1992, **4:** 1035–36.
5 Goodlad RA. Defective denominators, or will people never learn. *Gastroenterology* 1995; **108:** 1963.

Sir—Stanley Ewen and Arpad Pusztai's research letter[1] describing measurements of intraepithelial lymphocyte counts and mucosal thickness in rats in a short-term feeding experiment of potatoes transgenic for snowdrop lectin is unacceptable for the following reasons.

(1) For the intraepithelial lymphocyte counts, one essential group—rats fed with potatoes spiked with *Galanthus nivalis* lectin (GNA)—was omitted on the grounds that the authors claim to know that "dietary GNA or other lectins do not induce lymphocyte infiltration". This omission is improper and those data should have been provided.

(2) No control data are provided for rats fed on a normal laboratory diet so it is not possible to say what values are normal. The authors simply assume that anything found in group fed GM potatoes is abnormal.

(3) Were the assays done blind on coded samples?

(4) I am unclear as to what disease these markers are a surrogate. Intraepithelial lymphocyte counts are greatly increased in coeliac disease but in normal gut, a modest increase in their number is not known to me to be a marker for any pathological process. Also, what significance attaches to minor changes in mucosal thickness? If there is any evidence for pathological processes associated with these surrogate markers. Ewen and Pusztai should have cited it.

(5) In the statistical analysis there is no correction for "data dredging". The two measurements reported were not the test of a pre-existing hypothesis. They have been selected from an unstated number of comparisons. The probabilities need to be adjusted for the number of different comparisons made. This will almost certainly make them non-significant and the experiments therefore need to be repeated on a new group of rats.

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1 Ewen SWB, Pusztai A. Effects of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet* 1999; **354:** 1353–54.

Authors' reply

Sir—In their Oct 16 commentary Harry A Kuiper and colleagues discuss methods for testing the safety of GM foods. Our research letter only addressed the biological effects of GNA-GM potato diets on the morphology of the rat gut. However, since the safety testing issue has been raised we would like to respond.

According to the Rowett Institute's audit report the parent and transgenic lines we used were "substantially equivalent". At least for protein content this was confirmed in the alternative report put on the internet by the Rowett (not by Pusztai, as stated by Kuiper et al). All experimental diets were isoproteinic and isocaloric and supplemented with vitamins and minerals for optimal growth, and the food intake of all rats was the same. Although 6% protein is suboptimal, the diet was not protein-free, as it was in the paper cited by Kuiper et al. Since the rats were growing, to use emotional terms such as "starvation" is misleading. Furthermore, starvation reduces gut size and crypt length, precisely the opposite what we found. Since trypsin and chymotrypsin inhibitors have no effect on gut morphology, reference to them is irrelevant. The potato lectin is antimitogenic[2] so its effect should be a reduction in crypt cell proliferation rate and crypt size, not an increase. Moreover, GNA was selected for GM insertion because its effect on the gut is minimal so differences in lectin content, as an explanation for the biological effects reported, are also irrelevant.

Kuiper et al refer to "no consistency" in the changes. Is there any reason why changes in the different gut compartments should be the same? The ingestion of potatoes may indeed be associated with several adaptive changes, including caecal hypertrophy, but all the rats were given essentially the same potato diet containing the same starch component. Our study was about the effects of GM potatoes on gut morphology, not on their toxicology. The number of rats used (six per treatment group) was more than sufficient and most peer-reviewed, published papers on the biological and nutritional effects of similar diets (including over 40 from our laboratory) use three to four animals per group. Our controls were also sufficient. For both raw and cooked GM potatoes we used as controls both parent potato and parent potato supplemented with purified GNA, at the level expressed in GM potatoes. With this experimental design we could discriminate between the effects of the transgene product, the transformation, or the cooking and the interactions between these effects. The use of standard rodent diet of undefined composition as a control would have been inappropriate, and the effects of high-protein and low-protein diets would not have been comparable. We agree with Kuiper et al that attention should be paid to bioavailability and toxic effects. Kuiper et al cite their study done with a recombinant form of *Bacillus thuringiensis* toxin and not with toxin isolated from the GM-tomato. They themselves point out that since the stability, survival in the gut, and toxicity of a recombinant protein usually differ from those of the plant-gene product, the two forms can not be used for comparisons. Biotech companies, in their submissions to regulatory authorities, also rely on toxicity studies with *E coli* recombinants. We, by contrast, have been comparing the gene product from the GM plant with that of the non-transformed original.[3]

Indeed it would have been desirable for testing methods recommended by FAO/WHO and so on to have been used for GM food crops currently approved. Unfortunately, no such tests were required, carried out, or their results published. It is therefore surprising for Kuiper et al to state that "the data so far indicate GM-crops . . . that have been introduced into the environment do not differ from traditionally grown crops except for the inserted traits". Several traits having nothing to do with the transgene save the insertion have been found to differ in GM lines. The Monsanto analyses of glyphosate-resistant soya showed that the GM-line contained about 28% more Kunitz trypsin inhibitor, a known antinutrient and allergen.[4] GM-soya contained significantly less phyto-oestrogens than the parent lines.[5] Why is it that current GM crops need not be examined as thoroughly as the next generation?

We agree that "particular attention must be given to the detection and characterisation of unintended effects of genetic modification" but how will unknown toxins or allergens be found without first biologically testing the GM crop for toxicity? Writing of the sequence to be followed in future safety testing procedures, Kuiper et al say