

- 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 letter¹ 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.

Peter Lachmann

Academy of Medical Sciences, 10 Carlton House Terrace, London SW1Y 5AH, UK

- 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 isoproteic 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 antimutagenic² 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

"depending on the outcome . . . further toxicological and nutritional studies may be needed". The admission by people close to the decision-making committees of the European Union that the biological testing of GM food is needed appears to be an acknowledgment that previous safety testing could not have been rigorous enough.

Allan Mowat comments on the tissue fixation. This has long been a contentious topic. There may not be an ideal fixative but it is mischievous to suggest that the fixative upon which the whole of human histopathology relies could be responsible for different crypt-length measurements. The histology was part of a large series of synchronous physiological measurements on the same animal, demanding rapid tissue handling. Our animals were young (about 85 g) and, at that stage of development, the gut can easily be dissected from the mesentery to provide an unwashed, undistended histological sample at a constant distance from the pylorus. Orientation and plane-of-section problems were minimised by "splinting" tissue samples on card during standardised fixation. The jejunal crypt length of our 85 g rats has not varied and the data reported are similar to those obtained from 5000 jejunal crypts over the past 10 years. Rat intestinal crypt length depends on diet, animal supplier, and housing conditions, all of which are standardised in our rat colony, and the chemical composition of the potato diet was subjected to the exhaustive chemical analysis.

Intraepithelial lymphocytes (IEL) are thought to provide a surveillance function for damaged or virally infected cells, and any increase in the IEL population need not be limited to hypersensitivity reactions. Our young animals could not have been exposed to GNA previously, and any difference between groups is likely to be due to luminal factors in the diet. Moreover, IEL numbers are unaffected by lectin treatment.¹ The only difference in the diets is the presence of unidentified factors caused by genetic modification. Epithelial cell damage induced by the genetic modification cannot be excluded, and we would have studied it if our experiments had not been aborted. IELs averaged 7–11 per villus based on 48 villi counted, and we believe that our estimate is a useful indicator of unspecified immunological events of comparative value between groups. The suggestion that the low-protein content of the diet caused intestinal infection can be rejected; parasites were not evident in the

unwashed histological preparations and the lamina propria did not contain excess eosinophils.

Hyperplasia refers specifically to an increased cell number, frequently, but not necessarily, accompanied by increased mitotic activity although increased crypt mitotic activity is indeed present in our GM-potato fed animals. If, after 10 days of ingestion of GM potatoes villus pathology had been evident we would have described the damage. We reject the notion that potato or GNA lectin could have produced the changes that caused us concern.

Peter Lachmann's points (1) and (2) are addressed in our reply to Mowat. To point (3) the answer is yes, the measurements were done double-blind. With regard to point (4), Lachmann does not understand that the significant changes in mucosal indices represent another nail in the coffin of "substantial equivalence" on which the GM regulatory system is based. The differences in gut metabolic responses demonstrate that GM potatoes were not "metabolically equivalent", and this is important whether the changes are pathological or not. Increased epithelial cell proliferation in the colon is not regarded desirable and the high energy cost of small-intestinal hyperplasia may compromise growth and development.

In his point (5) Lachmann asserts that we had no prior hypothesis. Because the experimental design was obvious from the introductory part of our letter, to save space, a part of our sentence was omitted. Here it is: "It was thought that comparison of the histological parameters of the gut of rats fed potato diets containing either GM potatoes, or non-GM potatoes with or without being supplemented with GNA should give a clear indication whether GNA gene insertion had affected the nutritional and physiological impact of potatoes on the mammalian gut". Our table 1 clearly gives the statistical methods used and the number of comparisons. These methods were approved by independent statisticians. Lachmann says that the experiments need to be repeated. We would be happy to oblige. If our experiments are so poor why have they not been repeated in the past 16 months? It was not we who stopped the work on testing GM potatoes expressing GNA or other lectins or even potatoes transformed with the empty vector, which are now available. If Lachmann represents the view of the Academy of Medical Sciences on GM-food safety he should use his influence to

make funds available for the continuation of this work in the UK.

*S W B Ewen, A Pusztai

Department of Pathology, University of Aberdeen, Aberdeen AB25 2ZD, UK

- 1 Herzig KH, Bardocz S, Grant G, Nustede R, Folsch UR, Pusztai A. Red kidney bean lectin is a potent cholecystokinin releasing stimulus in the rat inducing pancreatic growth. *Gut* 1997; **41**: 333–38.
- 2 Kilpatrick DC. Isolation of a lectin from the pericarp of potato (*Solanum tuberosum*) fruits. *Biochem J* 1980; **191**: 273–75.
- 3 Pusztai A, Grant G, Bardocz S, Alonso R, Chrispeels MJ, Schroeder HE, Tabe LM, Higgins TJV. Expressin of the insecticidal bean alpha-amylase inhibitor transgene has minimal detrimental effect on the nutritional value of peas fed to rats at 30% of the diet. *J Nutr* 1999; **129**: 1597–603.
- 4 Padgett SR, Taylor NB, Nida DL, Bailey MR, MacDonald J, Holden LR, Fuchs RL. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *J Nutr* 1996; **126**: 702–16.
- 5 Lappe MA, Bailey EB, Childress C, Setchell C. Alterations in clinically important phytoestrogens in genetically modified herbicide-tolerant soybeans. *J Medic Food* 1999; **1**: 241–43.

Sir—Brian Fenton and colleagues' research letter (Oct 16, p 1354)¹ on the insecticidal *Galanthus nivalis* lectin (GNA) makes several questionable assertions. They state that the "distribution, abundance, and micro-heterogeneity" of structures recognised by GNA are "largely unknown". They then provide results which they claim "show that human white blood cells have many proteins that strongly bind to GNA". Several previous studies have reported examination of GNA binding to human proteins and tissues (including breast, Peyer's patches, kidney, brain, photoreceptors, plasma proteins, placenta, gallbladder, and several human-derived cell lines). Moreover, the binding of GNA to proteins from human white blood cells has been already been reported by Benallal et al.² The conclusions of the new work cannot be considered novel.

Fenton et al also argue that their results are relevant to the debate on genetically modified (GM) food, stating there needs to be "a greater understanding of the interactions of plant lectins and human glycoproteins before they can be safely incorporated into the food chain". However, plant lectins are already incorporated into the human diet. Not only are lectins naturally present in potatoes, lentils, beans, peas, tomatoes, and other crop plants but also many of these lectins bind to human glycoproteins. The first plant lectins were identified over a 100 years ago, and their binding to human cells, and