Royal Society Expert Reviewers of Pusztai Rat Experiments at the Rowett Institute from 1998-1999

Expert Reviewers 1-6 sent with mail to A. Pusztai on May 10 1999 and revised Expert Review sent to A. Pusztai on May 12, 1999

Reviewer 1 (Physiologist with Interest in Nutrition)

I can add little to what has already been written about the studies at the Rowett Research Institute but I have two small comments. The first concerns the diet fed by Dr Pusztai to his rats: Potatoes are a very inadequate diet for rats. Dr Pusztai was aware of their low protein content and made up some of the deficit with lactalbumin. However, the protein uptake of the rats was probably still well below the requirements. Dr Pusztai says nothing about minerals and vitamins, but potatoes would not provide rats with their requirements for these nutrients. It is possible that he gave the rats a mineral and vitamin supplement, but I can find nothing about this in the text. My personal comment - there appears to be no group of rats on a normal rat diet so it is impossible to tell how much the normal growth and development of the experimental animals was retarded on any of the potato diets.

I do not think the problem about transgenic foods will make much further progress towards solution by going over and over the experiments made at the Rowett Research Institute. This was pioneering work, but more research needs to be done. Since it is only plant foods that are involved I suggest that a herbivore e.g. the rabbit, might be a better experimental animal than the rat. Herbivores have a digestive system which is adapted to deal with the large amounts of plant foods that have to be eaten to provide for the animal's requirements. The staple food of rabbits is leafy plants. I do not know how easy it would be to produce a transgenic leafy plant, whether vegetable or not, but if this could be done dietary supplements would be unnecessary. If experiments on rats are to continue, with potatoes as the 'test' food, I suggest that the animals be fed some of the food normally fed to laboratory rats along with potato. One further issue is that nowhere in the papers I have received can I find any references to research in other European countries on GM foods for human consumption. If such research exists I suggest that reference be made to it, and if not, this might be mentioned.

Reviewer 2 (Quantitative Geneticist)

General

It is important to note that these experiments report the effects of one insertion of one transgene by one method, with the results tested by its effects on growth and immune responsiveness (recorded on one strain of one species at one age). General inferences can not be drawn about genetically modified foods, neither that they are harmful nor that they are not. This has to be dealt with on a case by case basis, taking account of the promotor, the inserted expressed gene, the background and so on. (To take an analogy: the fact that rats grew poorly when fed raw potatoes does not imply that potatoes should be removed from the human food chain.)

Reports of Audit Committee

The reports of the Audit Committee (Audit of data 21 Aug 1998, subsequently AuditD; and response to Project Coordinator 16 Feb 1999, subsequently AuditR) and the (preliminary, subsequently affirmed) statistical analysis by BIOSS (BIOSSp) are well taken, and I do not have substantial further comments to add. There is not convincing evidence of effects on growth, organ development or immune function of transgenic potatoes expressing GNA lectin. Nevertheless there are some indications, judged by numbers of statistically significant differences found, which need to be considered. Fundamentally, however, the experiments are defective in that major possible effects are confounded.

Confounding

There is a fundamental problem with the experiments, which becomes clear in the report of Dr Pusztai, the Programme Coordinator on 22 Oct (subsequently RPrCo), and the Audit Committee's response (AuditR). As Dr Pusztai points out, the transgenic potato strain (GNA-GM 74/2T) expressing GNA has a substantially different composition from its parental strain used in the experiments. It differed in protein content and also in other constituents (e.g. trypsin inhibitors). Steps were taken to correct the protein content of the diet by adding lactalbumin. Although the Audit Committee are satisfied this aspect is corrected, they raise the issue of the possible effects of other compositional changes, e.g. in glycoalkaloids (AuditR). In view of this, it is not possible to tell whether the effects, if any, of the modification on growth and other traits of the rats are due to the presence of GNA or to other factor, and whether the transgenic process was itself in any way involved. Fundamentally the experiment is rather simplistic in that major effects are confounded.

Removing confounding

It does not seem possible to disentangle these effects using the construct provided. To test whether the transgenic process per se was important alone, as seems to be inferred as a possible consequence of 'gene silencing...' (RprCo), it would be necessary to test potatoes transformed only by use of the promotor (CaMV35s in this case) connected to a non-functional gene. These would have to be insert lines selected such that there was no detectable effect on composition of the potato, e.g. protein content. To test whether any effects observed were due specifically to GNA synthesis, insert lines would

have to be assessed to find any with substantial GNA synthesis but not substantial effect on other protein production. It is clear that these are not a small or straightforward set of experiments, but confounded effects have to be separated if inferences are to be drawn.

Animals

No information is provided in the protocols on the rats used, for example strain or any particular features.(This may or may not be important, but inferences have been drawn by the non-scientific community from a single population of a single species)

Animal assignment and data analysis.

No information is given on the way in which animals were allocated to treatment groups. The analysis is based on the assumption that test animals were uncorrelated (BIOSSp); this implies, for example, that litter-mates were not reared on the same diet. This can be dealt with in the analysis by fitting litter effects in the statistical model, and checking whether they are important. It is noted that animals were pair fed in some experiments; this seems not to have been taken into account in the statistical analysis. Importantly, attention is drawn in the statistical analysis (BIOSSp) of the non-random assignment to ELISA plates.

Statistical significance.

The experiment was not set up with any particular hypotheses as far as the many organs were concerned. it is essentially 'look see' and so multiple statistical tests have had to be made. The weights and proportions of different organs of animals are correlated. Hence as pointed out (BIOSSp), the tests are not independent of each other, and the distribution of numbers of 'significant' tests is not known. Under the null hypothesis, the probability a significant result (assuming the correct analysis was used) is 5%, and the expected number significant in n tests is 0.05n. With independent tests the distribution of the number significant is binomial, and therefore in itself testable; but this can not be done in this case. Hence we do not know the probability of finding the observed number of significant results, except but it is much larger than if the tests were independent.

Reviewer 3 (Physiologist With Knowledge Of Nutrition)

I found the data impossible to review in the usual sense since proper descriptions of methodology are missing but my overwhelming impressions are of extremely poor experimental design, for whatever was intended to be the outcome of the work, chaos and confusion, with hopelessly confounded issues. The main problem in the experiments is the diet of the rats. The crude protein content in the four experiments was approximately 5, 5, 7.5 and 10% which is considerably lower than that of commercial rat food, the formulation of which is based on evidence of nutritional requirements. Although the rats continued to grow, at these marginal (10%) or much too low levels (5 and 7.5%) of protein intake, there was the expected considerable variation between animals and tendencies for some organs to be affected differently.

Immune responsiveness might also be expected to be highly variable but the statistical design (see the BioSS report on the sample layout in the plates) for these studies seems so flawed that they may appear useless (Professor John Waterlow FRS is the expert on the effects of protein under-nutrition and might comment on this area). Against this background there is possible variation in nutrient composition of the potatoes used in the study (some of the variation was apparently adjusted by adding a bovine lactalbumin preparation) and the lectin content of the GM potatoes. Any chance of producing proper comparisons, further confounded by changes in the basic diet between experiments, is compromised. Another confounding factor is the use of raw potato, well known to be poorly tolerated by rats. I discount all attempted comparisons based on raw potato diets.

Therefore I see no evidence that gives me any cause for concern beyond present knowledge and known safeguards either of the effect of genetic manipulation on nutritional content (which can be determined with high levels of precision by chemical analysis and by experiments using properly formulated diets for long periods in appropriate numbers of animals) or of incorporating lectins of known toxicological properties into potatoes. I also see no need for further work in this specific area since any GM potatoes destined for the market would be tested by appropriate methodology at the time.

Reviewer 4 (Expertise in Statistics and Clinical Trials)

Introduction

This report deals mainly with aspects of the design and statistical analysis of the experiments described by Professor Pusztai himself. Some references are made to the report by the specially convened audit committee. The committee's report is the only source of information on some experimental details of relevance and Professor Pusztai acknowledges this. There is at least one central issue (dietary equivalence) on which Professor Pusztai and the committee disagree and it is unfortunate that the review was not conducted by a group consisting of experts outside the Rowett Research Institute. The independent statistical report is summarized and evaluated.

Overview

The general aim of the work commissioned by SOAFED is set out but there are no detailed specifications giving prior hypotheses to be tested, particularly about the possible direction and magnitude of any anticipated differences. These considerations are important in estimating required sample sizes especially - as here - in evaluating a series of experiments giving rise to scores of potential comparisons some of which will indicate differences that have in fact arisen by chance. However, the numbers of animals in different experiments appear to have been decided arbitrarily and may not have been adequate to allow for the different sources of variability likely to have been encountered. (Experiment D211, Appendix 3 of the audit committee's report, though not one of the four leading experiments in Professor Pusztai's report, indicates – for example - that there were 3 animals in one group and 2 in the other.) It is true that there were rather more statistically significant results than might have occurred by chance but to the extent that their interpretation was post hoc, conclusions as to their possible biological significance are easy to suggest but may be difficult to justify (except where they are supported by other, independent data). More convincing than simply the number of significant results

would be evidence of consistency within and between experiments which, as the Biomathematics and Statistics report points out, is lacking. Presumably dose-response effects in the results of the immunological experiments would have been expected but are not apparent. Some of these points are made in more detail later. In considering the results of most if not all the experiments, the "blindness" or otherwise of technical staff taking part is an important consideration. This may be especially so where organ weights were involved since dissection and separation of parts of the alimentary tract could have been influenced by knowledge of the experimental groups to which the animals were assigned. Biased assessments may have been one reason for the larger number of significant results than expected.

Experimental data

The tabular material is not always very clearly presented. Table 1 appears to show that 74/2T* and 71/1+ were used for comparing parent and GNA transgenic effects, the former based on raw material and the latter on boiled material. It seems that 74/2T should also have an * for comparison of parent and transgenic material in baked material. It appears that "parent" is synonymous with "control" (which is particularly unclear from the audit committee's report). Concentrations of several constituents were not determined (Tables 1 and 2), particularly in the case of 74/2T which was used in trials D237, D242 and D249. Professor Pusztai says the results clearly show that the content of some or all of the constituents in GM-potatoes are significantly different from those of their respective parent lines. This may be true for N and protein in 74/2T but cannot be assessed for other constituents because of missing data. Results for total N, NPN, N after dialysis and protein are not obviously different for 71/1 whereas there may be differences for starch and glucose polymers. Whether any of the differences are statistically significant cannot be determined without information about numbers and measures of dispersion (e.g. standard deviations). Professor Pusztai's conclusions are not easy to follow but it is possible that there were differences between some of the GNA-GM potato lines and their parent lines. If so, there may be some substance to his claim that the audit committee's statement that the lines were "substantially equivalent" was not entirely justified but, equally, the committee may be correct. This crucial point appears to be unresolved. The audit committee points out that glycoalkaloid content of different diets was not measured, introducing one of several potentially confounding factors.

Requirement that diets often needed supplementation clearly makes many comparisons in the feeding studies difficult if not impossible to interpret with confidence. Is it accepted that this would be less important in short-term trials than in the long-term experiment? Protein imbalance was particularly acute in the long-term study because the tuber line available in sufficient amounts contained almost 20% less protein than its appropriate parent line.

In Table 2, what is actually being compared, by what tests and what the significance levels were (other than simply being < 0.05) is not clear. The multiple comparisons made illustrate the difficulty of picking out differences that may indicate real biological significance. Conversely, no information on significance levels is provided for Table 3 so that the conclusion that the behaviour and binding of "potato GNA" in the gut on feeding GNA-GM-potatoes was similar to that of "snowdrop GNA", fed as part of a diet containing parent potatoes spiked with GNA, is not necessarily justified.

Experiment D227 was considered as preliminary. No fewer than seven diets were tested, resulting in a confusing mixture of possible comparisons involving assessment of added GNA and of transgenic lines

either with each other, with raw or boiled parent potatoes or with LA controls. The main outcome presented is that growth was significantly reduced on boiled potato diets and to an even greater extent on diets containing raw potatoes, but the full range of comparisons potentially arising from the seven diets involved is incomplete. What did the other results show? The Table shows no data if differences were not significant. It illustrates the general point that results and p values should have been given whether or not these achieved a conventional level of statistical significance since, with the small numbers of animals involved, trends as well as levels of significance are necessary to assess general consistency. Professor Pusztai points out that the data from D227 were in possession of the audit committee but not included in their report.

Experiment D242 had to use second generation tubers of GNA-GM-potatoes from 71/2T and a mixed control consisting of 19% 71/2T and 81% 74/2G control lines because of the short supply of 71/2T control potatoes. This was not Professor Pusztai's fault but introduces a potentially significant complication into the interpretation of the experiment's findings. Supplementation with lactalbumin was also necessary. Again, seven diets were tested. GNA, whether added to the diets or expressed in the transgenic line 71/1, had no significant effect on overall weight or weight change compared to parental potato lines. However, because of differences between final body weight and empty body weight it was suggested that digestion and absorption of nutrients in transgenic diets was retarded by comparison with ordinary potato diets. As before, only some of the many comparisons enabled by the seven diets tested are shown, with missing data where differences were not conventionally significant and, consequently, no indication of whether trends for other organs were seen in these cases. There is no comment on whether the apparent reversal in the case of the thymus and gastrocnemius muscle can be explained and be of any biological significance. It is quite possible that these findings simply reflect chance differences in an unexpected direction. It is not only differences between transgenic and parent line diets that matter but also - presumably - any dose-response trends for any of the diets tested. t-tests compare two means (either from paired or unpaired series) which is not the situation indicated in Tables 6 and 7 so there is an ambiguity if not an error to be considered. Where more than two means are tested, analysis of variance (A.V) would be appropriate (as well as tests for trend within each diet).

Experiment D237 was the only long-term experiment carried out with GNA-GM-potatoes. It is considered to have been a preliminary study to enable dietary modification in further experiments to be carried out in ways that would provide "ideal conditions". It was practically impossible to formulate isoproteineic diets and lactalbumin supplementation (see above) was necessary. Three diets were tested in one part of the experiment and two in another. Interpretation of results requires reference to Appendix 8 of the audit committee's report and also depends on the written summary without data by Professor Pusztai on weight changes. The only significant difference found was in liver weight. In contrast to the previous studies in which there was apparently partial liver atrophy in rats given transgenic potatoes, both absolute and relative weights of the liver of the animals on transgenic diets were significantly increased, suggesting that it may have been the dietary level "of the high quality lactalbumin in the transgenic diet that might have caused a general increase in the organ weight of the rats on this diet". No significant differences between transgenic and parent lines were found in lymphocyte responsiveness.

Experiment D249 was in progress while the audit committee was deliberating. The GNA-GM-potato line 71/1 and its corresponding control line were used. A high dietary level of lactalbumin was deliberately used to see whether differences in organ weight and lymphocyte responsiveness in previous short-term experiments with diets containing low levels of lactalbumin could be over-ridden. No significant differences were found in the growth rate of the animals. However, empty body weight of the transgenic-fed rats was significantly higher than in the parent control group. Despite the high lactalbumin concentrations, there were "still" significant differences in organ weights. Based on relative wet weight, spleen weight was slightly higher, liver weight somewhat lower (both $p< 0.05$) and brain weight lower ($p< 0.001$) in rats fed raw transgenic potato compared with those fed unmodified potato. Professor Pusztai concludes that GNA in GNA-GM-potato diets appears to show functional equivalence to "snowdrop GNA" in parent potato diets spiked with GNA. He also claims that the presence of GNA-GM-potatoes caused some slowing of the digestion and absorption of nutrients in the gut and that GNA-GM-potatoes significantly reduced lymphocyte responsiveness to mitogenic stimulae after ten days.

Statistical report

The independent statistical report is of a high and reliable quality. It points out that the symmetrical arrangement on the plates used for immunological assessment means that concentration was confounded with well position and there was also some confounding of diet effects with between-plate differences. The report also points out that not all seven diets were included in all experiments and draws attention to the additional lactalbumin diets. In experiment D249, immunological assessment was carried for only three or four rats on each diet though on spleenocytes for all rats. In experiment D242, pooling of blood samples from pairs of rats on the same diet reduced the number of replicates to three. The report refers to one-way analysis of variance (AoV), apparently carried out by the statistical group and not by Professor Pusztai himself (see above). AoV is the appropriate method for many of the experiments. The report demonstrates more significant contrasts than would be expected if diets were identical and significant results occurred only by chance (Type 1 error). However, the tests were not all independent of one another and include unexpected as well as more expected findings. There is also the possibility (see above) that biased measurements (very probably quite unconscious, if they occurred) led to some significant differences. The report rightly comments that no consistent pattern of significant results was found and that there was no systematic relationship between response and mitogen concentration in the immunological studies. There was an intrinsically low chance of obtaining significant results, given the variability of the measurements concerned. There was "no easily interpreted pattern of effects". Power for the immunological analyses was even lower that for organ weights because of greater variability and the small number of animals used.

Conclusion

In the form currently available, Professor Pusztai's results provide no reliable and therefore no convincing evidence of adverse (or beneficial) effects either of lectins added to unmodified potatoes or of genetically modified potatoes on the growth of rats or on their immunological function.

In summary, the main reasons are:

(i) the absence of specific (as opposed to general) hypotheses to be tested (ii) small numbers of animals used in experiments testing several diets and resulting in multiple comparisons (iii) confusing

experimental design (iv) uncertainty as to whether there was or was not substantial equivalence between unmodified and modified potatoes (v) actual or possible confounding due to dietary differences (unmeasured in the case, for example, of glycoalkaloids) or dietary enrichment to meet Home Office and other requirements (vi) possibly biased measurements (vii) apparently inappropriate statistical techniques (viii) lack of consistency of findings within and between experiments.

With hindsight, Professor Pusztai attempted to do too much with the resources at his disposal. It is regrettable that he has still not submitted his work for publication and the peer review that might have enabled him to deal with some of the shortcomings involved. There is, of course, also the question of how far results in animals can be used to suggest effects in man.

[In terms of clinical trial design, conduct and analysis, the Royal Society would be justified in concluding that Professor Pusztai's work provides no evidence of harmful effects of added lectins or of genetically modified potatoes. At the same time, it would be of the greatest importance to avoid the error made by others over BSE - that no evidence of effect is the same as evidence of no effect. In other words, it is not possible to conclude that Professor Pusztai's work can be taken to indicate that there is no possible cause for concern. Clearly, properly designed experiments with clear prior hypotheses, adequate statistical power, meticulous conduct and appropriate statistical methods for analysing results are likely to be the only way in which it may be possible to demonstrate that the likelihood of harmful effects is so small that positive reassurance becomes possible, though even then a very remote chance of harmful effects could not be completely ruled out.]

ALTHOUGH ALL REVIEWER'S COMMENTS HAVE BEEN COPIED TO DR PUSZTAI TO ALLOW HIM THE OPPORTUNITY TO RESPOND, THE REVIEWER ASKED THAT THE FINAL PARAGRAPH (IN SQUARE BRACKETS) BE RESERVED FOR THE WORKING GROUP ONLY.

Reviewer 5 (Nutritionist)

Terms of reference:

- to consider the data generated by experiments carried out as part of SOAEFD Fund Project RO818 regarding toxicity of GM potatoes and reach an opinion on whether the data provide cause for concern relating to: 1. The specific GM material studied and 2) GM food material in general.

Experiments

The series of studies represent a preliminary exploration to try to develop an approach to determine in the short (10 days) and longer term (110 days) whether a particular foodstuff (a modified potato) fed to rats, alone or in combination, was associated with changes in a number of variables. As the experience of the investigators developed, there were changes to the structure of the experiments resulting in a range of experimental designs. The outcomes of interest included changes in the body weight of the rats and in the weight of individual organs, together with measures of immune responsiveness. Based on the statistical analysis of the experimenter, the audit group and independent statistical opinion it is agreed that some of the results were statistically significantly different in those animals fed diets which contained GM products. These differences were more common than would have been expected by

chance. The unusual results did not fall into a readily discernable pattern. It is not possible to say whether the unusual results in these experiments were simply the result of chance, due to limitations in the experimental design, a consequence of an effect of the material under test, or a combination of all three.

Results

The results of this series of studies are best described as equivocal. The food material can not be considered to be exonerated from association with any (adverse) change. However, on the basis of this evidence a justifiable basis for concern of relevance to health has not been sufficiently demonstrated. There is a justifiable basis for concern that the material cannot be said to be without effect, and therefore there is the need for further studies. The objective of these studies would be to take the preliminary observations and seek to reproduce the findings, to determine and explain the nature of any differences observed.

The only way to clarify the current situation would be to use the results of the current investigation as the basis for further studies in which clearly defined hypotheses are tested, focussed on the specific differences already identified. It would be important to ensure that these studies had sufficient statistical power (numbers in each experimental group in relation to the variability in individual response) to come to a clear conclusion. It would also be important to take adequate account of important biological variables which would include: a) the age and the susceptibility of the animals, b) the wholesomeness and completeness of the entire diet, c) the specific targets for any hypothesised damage.

Conclusions

It would not be possible to generalise from the results of these experiments to the safety or otherwise of all GM foods under all conceivable circumstances. Therefore, there is the need for an agreed, acceptable process for testing the safety of food and food ingredients, which is unlikely to be the same as that used for testing the safety of pharmacological compounds. It is unlikely that a form of testing can be found which will ever give an absolute assurance of safety. There is the need to recognize the likelihood of a variable response amongst individuals and within the same individual depending upon their physiological state, lifestyle, and the rest of the diet. Any assurance is likely to be for the foodstuff within the normal limits for which that foodstuff is likely to be consumed, or is habitually consumed over extended periods of time.

Reviewer 6 (Nutritionist with Expertise in Immunology)

Background

Lymphocytes are transformed into dividing lymphoblasts when they are stimulated by specific antigens. This represents one of the early events in cell-mediated immune reactions in the body, and it can be mimicked in vitro by the isolation of lymphocytes which are then cultured in the presence of a mitogenic stimulus or foreign cells.

Certain plants lectins such as phytohaemagglutinin (PHA) and concanavalin A (ConA) will stimulate lymphocytes to divide in vitro, a phenomenon first described about 30 years ago. Lymphocyte proliferation can be measured by a standard technique which involves the addition of a radiolabelled nucleotide to the culture medium and the determination of its incorporation into the DNA of the cell. The response thus measured depends on several factors including the purity of the lymphocyte preparation, the numbers of T cells and accessory cells, and the optimal concentration or reagents. In view of the complexity of the system it needs a stringent protocol and thorough optimisation if reproducible results are to be achieved.

Experimental

The work reported by Dr Pusztai describes three experiments. In the first experiment (D214), which was long-term, rats were fed for 110 days with a diet of cooked potatoes to which Con A was added. At the end of the experiment lymphocyte responsiveness was tested with the same mitogen in vitro. Not surprisingly, lymphocytes from rats fed potatoes with added Con A were less responsive than those from rats fed potatoes alone. This was not the case when the diet consisted of lactalbumin-starch instead of cooked potatoes. PHA was claimed to give more consistent results than Con A in the in vitro test. In the second experiment (D242), which was short-term, rats were fed with cooked or uncooked potatoes with added snowdrop lectin, GNA, or with transgenic potatoes which expressed GNA for 10 dayss. Lymphocytes from rats fed uncooked potatoes responded well to Con A in the lymphocyte proliferation test, whether the diet was spiked with GN or not. In contrast, rats fed cooked potatoes spiked with GNA showed reduced lymphocyte responses. Lymphocytes from rats fed transgenic potatoes showed very low responses to Con A irrespective of whether the potatoes were cooked or uncooked.

In the third experiment (D237), which was again long-term, rats were fed transgenic GNA potatoes for 110 days. Lymphocyte responsiveness was much reduced when rats were fed cooked potatoes, cooked potatoes with added GNA, or cooked transgenic potatoes. In other words, no additional effect of transgenic potatoes was detected over and above that of the cooked potatoes alone.

A common thread runs through the results in that there was an indication that the unusual diet of cooked potatoes, and of transgenic potatoes, might have caused a reduction of the immune response. The reduction is akin to the well known stress-induced reductions of the immune response. A similar point was acknowledged by Dr Pusztai in his report of 22 October 1998 but a different type of study would be needed to resolve the causal factor(s).

Interpretation

Clearly, the interpretation of these results is problematical for several reasons. No single experiment was duplicated; considerable variation occurred between individual animals as indicated by the large variances raising doubts about whether the tests had been adequately controlled at a technical level; the statistical analyses were based on the comparison of rations (stimulation index) - all factors that would urge caution in the interpretation of these data.

At worst, the interpretation of these preliminary findings has been alarmist. This reviewer would not have recommended publication of the results because of the incomplete nature of the experiments, the

need to establish an experimental design which would clarify, with stringent controls, the sequence of any change in immune responses during the feeding trials, and the lack of statistical rigour.

Recommendation

It is recommended that the findings prompt further investigation by the Scottish Office and the Ministry of Agriculture, Fisheries and Food to establish their validity and utility in the regulatory process for novel foods and processes. In view of the public interest in this case it is further recommended that, when completed, the work should be peer reviewed and published both in a suitable scientific journal and a readily accessible outlet, and placed on the World Wide web. This would provide an opportunity for the international scientific community and the public at large to have access to the information.

Revised Version of one of the Reviewers from 12. May 1999

Dear Professor Pusztai,

I have pasted in a revised review from one of the reviewers - you have already been sent the first draft of this report, but it has since been revised. Please let me know if you receive this email.

Best Wishes, Rebecca Dr Rebecca Bowden Senior Manager, Science Policy
The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG

Transgenic potatoes and their alleged effects on immune responses in the rat (revised 12 May 1999)
Experiments of Dr Arpad Pusztai (SOAEFD flexible fund project RO 818)

Background

Antigen-reactive lymphocytes are transformed into dividing lymphoblasts when they are stimulated by specific antigens. This represents one of the early events in antigen-specific cell-mediated immune reactions in the body, and it can be mimicked in vitro by the isolation of lymphocytes which are then cultured in the presence of a mitogenic stimulus or foreign cells.

Plant lectins are normal components of some human diets. Certain lectins such as phytohaemagglutinin (PHA) and concanavalin A (Con-A) will stimulate whole populations of lymphocytes to divide in vitro, a phenomenon first described about 30 years ago and widely used as a surrogate test for lymphocyte reactivity. Lymphocyte proliferation can be measured by a standard technique which involves the addition of a radiolabelled nucleotide to the culture medium and the determination of its incorporation into the DNA of the cell. The response thus measured depends on severalfactors including the purity of the lymphocyte preparation, the numbers of T cells and accessory cells, and the optimal concentration of reagents. In view of the complexity of the system it needs a stringent protocol and thorough optimization if reproducible results are to be achieved. The results are expressed as a DNA stimulation index derived from samples tested with and without a stimulating mitogen. The resulting ratio has to be evaluated by specific statistical procedures (eg logarithmic transformation) to determine the significance of differences.

Experimental

The work reported by Dr Pusztai describes three experiments. In the first experiment (D214), which was

long-term, rats were fed for 110 days with a diet of cooked potatoes to which Con A was added. At the end of the experiment lymphocyte responsiveness was tested with the same mitogen in vitro. Not surprisingly, lymphocytes from rats fed potatoes with added Con A were less responsive than those from rats fed potatoes alone, possibly because of receptor occupancy by dietary Con A. However, this was not the case when the diet consisted of lactalbumin-starch instead of cooked potatoes implying that the previous lack of response was due in some way to the diet of potatoes. Another mitogen, PHA, was claimed to give more consistent results in the in vitro test.

In the second experiment (D242), which was short-term, rats were fed with cooked or uncooked potatoes with added snowdrop lectin, GNA, or with transgenic potatoes which expressed GNA for 10 days. Lymphocytes from rats fed uncooked potatoes responded well to Con A in the lymphocyte proliferation test, whether the diet was spiked with GNA or not. In contrast, rats fed cooked potatoes spiked with GNA showed reduced lymphocyte responses. Lymphocytes from rats fed transgenic potatoes showed very low responses to Con A irrespective of whether the potatoes were cooked or uncooked.

When this experiment was repeated (D249) using a high protein diet supplemented with lactalbumin, splenocytes (rather than peripheral blood lymphocytes) from rats fed raw potatoes responded to Con A at the lowest doses and to PHA at the highest doses, further suggesting that optimisation of the assay system was inadequate. When the raw potato diet was supplemented further with GNA, or replaced by transgenic potatoes, splenocyte responsiveness was reduced for both antigens at all doses tested even though the dietary protein level had been enhanced. In the third experiment (D237), which was again long-term, rats were fed transgenic GNA potatoes for 110 days. Lymphocyte responsiveness was much reduced when rats were fed cooked potatoes, cooked potatoes with added GNA, or cooked transgenic potatoes. In other words, no additional effect of transgenic potatoes was detected over and above that of the cooked potatoes alone.

Interpretation

One interpretation of these findings would be that the unusual diet of potatoes rather than the presence of a transgene caused a reduction of mitogen-sensitive lymphocyte responsiveness. Stress-induced reductions of the lymphocyte responsiveness has been well established. Dr Pusztai hinted about this possibility in his Report of 22 October 1998. It is impossible from the present study to claim that transgenic potatoes caused a reduction in the immune response in rats. The tests were not statistically significant and would need to be carried out with a different antigenic stimulus in vitro to establish whether lymphocyte responsiveness was reduced. Much more sophisticated analysis would be needed to determine whether there were changes in cell populations after feeding potatoes with our without added lectin or transgene. There was no evidence that the transgene had a different effect from added lectin. The investigator did not even report the numbers of lymphocytes in circulation after treatment which was an elementary omission.

Clearly, the interpretation of these results is compromised by other experimental weaknesses. No single experiment was duplicated; considerable variation occurred between individual animals as indicated by the large variances raising doubts about whether the tests had been adequately controlled at a technical

level; and the statistical analyses were based on the comparison of ratios (stimulation index) which cannot be used for the test adopted (t test). All these factors urge that the data are unsafe.

At worst, the interpretation of these preliminary findings has been alarmist. This reviewer would never have recommended publication of the results because of the incomplete nature of the experiments, the need to establish an experimental design which would clarify, with stringent controls, the sequence of any changes in immune responses during the feeding trials, and the overall lack of statistical rigour.

Recommendation

It is recommended that the findings prompt further investigation by the Scottish Office and the Ministry of Agriculture Fisheries and Food. They would need to fund experienced immunotoxicologists to establish a valid and proven procedure that would be useful in the evaluation of novel foods and processes for regulators. In view of the public interest in this case it is further recommend that, when completed, the work should be peer reviewed and published both in a suitable scientific journal and a readily accessible outlet, and placed on the World Wide Web. This would provide an opportunity for the international scientific community and the public at large to have access to the information.