

berry scotland

Notes from the

Berry Research Meeting

held on 21st February 2003
at the West Park Centre,
University of Dundee

Sponsored by the Scottish Executive and Scottish Enterprise

The programme:

- 9: 15 Welcome.
Professor Mike Lean
- 9: 20 Introduction.
Ms Julie Beattie
- 9: 30 Soft fruit breeding at the Scottish Crop Research Institute.
Dr Rex Brennan
- 9: 50 Quality, Health & Nutrition Programme at Scottish Crop Research Institute.
Dr Derek Stewart
- 10: 10 Berry related research & work on salicylates at the Rowett Research Institute.
Dr Garry Duthie
- 10: 35 ESR methods at the Rowett Research Institute.
Dr Donald McPhail
- 10: 55 Coffee
- 11: 15 Berry research at Glasgow University, Plant Products and Human Nutrition Group.
Professor Alan Crozier, Professor Mike Lean
- 11: 35 Vascular disease studies.
Professor Jill Belch
- 11: 55 Cancer studies.
Professor John Hayes
- 12: 15 Questions
- 12: 30 Lunch
- 1: 30 Discussion chaired by Prof Mike Lean.
Ways forward on key areas of interest raised by the group:
1. Identification of compounds that give antioxidant capacity in each species and compounds that are biologically relevant
 2. Gene-phenolic compound interactions and alteration of gene expression by berry phytochemicals
 3. Properties of berry components in relation to cancer & heart disease and mechanisms of action
 4. Absorption, metabolism, bioavailability, and *in vivo* properties of berry components
- 3: 30 / 4: 00 Finish.

Welcome

Prof Mike Lean (Department of Human Nutrition - University of Glasgow)

Introduction

Julie Beattie (Berry Scotland Programme & University of Dundee)

Aims of the Berry Scotland Programme: to promote an increase in the consumption of Scottish soft fruit for the benefits of population health and the Scottish berry industry. Figures from the National Food Survey show that Scottish consumption of soft fruit stands at around 20g per week on average. Compare with Finnish consumption of 125g per week on average.

Gaps in our knowledge on health benefits of berries were highlighted which also reflected the types of research questions raised by the group on returned forms.

Scientific expertise present at the meeting included a wide range of disciplines including molecular biology, biochemistry, plant science and fruit breeding. People with access to animal models and human trials, experts in human diseases, food processing, flavour and dietary assessment, behaviour and marketing. The purpose of the day was to encourage sharing of knowledge and methods for analysis of bioactive compounds in berries. Also to identify key questions for future research collaborations and how these ideas could be moved forward.

Speakers:

Dr Rex Brennan – Scottish Crop Research Institute

Soft Fruit Breeding and Genetics at SCRI

1. Soft Fruit Breeding.

Breeding programme focuses on *Rubus* (raspberries, blackberries and hybrid berries) and *Ribes* (blackcurrants). Also includes *Fragaria* (strawberry). Minor berry fruits that are available at SCRI but are not part of a breeding programme at present are *Vaccinium* spp., *Amelanchier*, *Hippophae*.

2. Genetic Resources.

SCRI hold large germplasm collections of many species including wild accessions from around the world and historical and current cultivars from the UK.

3. The fruit breeding programmes at SCRI are commercially funded.

Glaxo Smith Kline fund the *Ribes* breeding.

Funding for *Rubus* is via an industry consortium (under negotiation). Breeding is considered too “near-market” to receive government funding.

4. Genetic work includes:

Marker development, linkage mapping, deployment strategies for marker assisted breeding e.g. root rot resistance, development of mapping populations, introgression of new resistance genes, phenotyping of genetic resources at whole plant and molecular levels.

5. *Ribes* breeding.

SCRI cultivars occupy 95% of UK and 50% of global acreage.

Ben Hope = first gall mite resistant processing cultivar and Ben Gairn = first reversion virus resistant cultivar.

Through a wide genetic base, can respond to new priorities and requirements. Currently the emphasis is on fruit quality eg ascorbic acid content and anthocyanin levels.

6. *Rubus* breeding.

SCRI cultivars are grown across Europe and North America, the main one in the UK being Glen Ample. Now new material for the fresh market is important. The blackberry programme has selections under commercial trials and there are advanced material tolerant to root rot (a first).

Can essentially breed any characteristics required into the fruit.

7. Raspberry acreage has decreased over the last 20 years, blackberry acreage has increased and covered production under tunnels has increased. Protected cropping will have implications for the phenolic content of the fruit and this is about to be investigated by SCRI.

8. It takes at least 11 years from initial crossing to cultivar release.

Breeding is the key mechanism for delivery of SCRI's fundamental science programme to the market place using a multidisciplinary approach (molecular geneticists, breeders, entomologists, virologists and physiologists). There is an urgent need for new sources of pest and disease resistance, quality and adaptation to new cropping systems.

9. In *Rubus*, quality and sensory characteristics are most important now, eg fruit size, colour, juice yield, anthocyanin content, flavour, aroma.

10. In *Ribes*, priorities for blackcurrant breeding have changed. Up to the mid 1990s, resistance and tolerance to disease was important as was yield and juice quality. Since the mid 1990s, high vitamin C, low acidity, phenolic compounds, disease resistance, high delphinidin content and sensory attributes are important.

11. Ascorbic acid in blackcurrant.

Levels vary from 50 to over 300 mg per 100g in different cultivars. Most steps in the biosynthetic pathway have been described but heritability of high ascorbic acid and site of biosynthesis are still unclear. There are strong environmental effects on ascorbic acid content and there are opportunities for breeding and marker development.

12. Ascorbic acid, anthocyanin and antioxidant levels vary across *Rubus* and *Ribes* species. A *Rubus* anthocyanin devoid mutant exists.

13. Other nutrients present: GLA and other fatty acids, ellagic acid.

14. New crops for commercial processing include aronia and seabuckthorn.

15. Future directions.

Characterise germplasm collections at SCRI

Improve phenotyping methodologies

Selection of higher quality phenotypes

Identify and exploit markers linked to traits – especially fruit related characteristics

Increased utilisation of SCRI fruit cultivars and research both within the UK and overseas.

Dr Derek Stewart – Scottish Crop Research Institute

Quality, Health & Nutrition Programme: Bioactive compounds in soft fruit.

1. SCRI fruit-related projects:

a) Biodiversity in the antioxidant status and composition of *Rubus* and other soft fruit germplasm.

b) Antioxidant compounds from novel sources of anthocyanin rich and anthocyanin depleted fruit species.

c) The bioavailability and bioefficacy of soft fruit antioxidants.

d) Strategies to improve ascorbic acid content.

e) Cultivation and breeding of Northern *Rubus* species.

f) Domestication of Northern berries.

g) Novel packaging strategies for maintaining soft fruit quality e.g. have managed to keep strawberries edible for 60 days. This is now the subject of a LINK grant.

2. The antioxidant capacities of various plant-derived foodstuffs (in micromoles TEAC per gram):

raspberry 12-23 (wild *Rubus* 1-47)

strawberry 8-19

blackcurrant 12-60 (wild *Ribes* 9-190)

blueberry 19-45

orange 7.5

red wine 10-18

white wine 2-5

3. The contribution of vitamin C to the antioxidant capacity:

This varies enormously from a very low level in blueberry to around 10% in raspberry, around 40% in strawberry and just over 80% in blackcurrant.

4. The anthocyanin content of selected fruits (in mg/kg):

blackberry 1150
blackcurrant 1300 +/-4000
blueberry 825 +/-4200
black raspberry 1700 +/-4277
red raspberry 100 +/-600
strawberry 150 +/-350
plum 20 +/-250

5. Soft fruit types of anthocyanins:

Pelargonidin and pelargonidin-3-glucoside mostly in *Fragaria*

Cyanidin, cyanidin-3-glucoside and cyanidin-3-galactoside are mainly in *Rubus*

Delphinidin and delphinidin-3-glucoside are mainly in *Ribes*.

6. The antioxidant capacity (whether measured by FRAP or TEAC) correlates well with the phenol content.

7. SCRI Collaboration: Cultivation and domestication of northern *Rubus* species: Northberry.

Cultivars of Northern *Rubus* species for commercial production will be developed.

Testing of cultivation among farmers involved in the project.

Production of parthenocarpic cloudberries and arctic brambles and determination of quality parameters, particularly the health related compounds.

8. There is a high degree of variation between the antioxidant capacities of berry juices both within and between species. Blackcurrant and blueberry generally are higher in antioxidant capacity. One wild *Ribes* species sticks out with an extremely high antioxidant capacity.

9. Vision/ Future:

Bridging the gap between the plant chemistry and biomedical science.

Soluble antioxidants have been extensively characterised but cell wall antioxidants must now be analysed since their activity may be exerted in the intestines and colon (potential for animal studies – effects of cell wall material on gut).

Ultimately, ideal characteristics can be defined for future fruit breeding.

10. Is there an RDA of antioxidants?

11. Future SCRI bioactive compound research:

Biomimetic efficacy testing – *in vitro* LDL oxidation inhibition studies using *Rubus* extracts.

In vitro digestion studies.

Inheritance of antioxidant capacity.

Cell line and *in vivo* analysis of the effect and metabolic fate of *Ribes* / *Rubus* derived bioactive compounds.

Dr Garry Duthie – Rowett Research Institute

Background

The Rowett Research Institute aims to conduct research at the forefront of nutrition, defining how nutrition can prevent disease, improve health, and enhance the quality of food production in agriculture. Within the

context of this mission statement, the remit is to advance understanding of the molecular and physiological mechanisms underlying nutrition in animals and man and to identify how this can improve sustainable agriculture, food quality and human health.

The research is focused on developing an understanding of the biological activity of specific nutrients at a cellular level. In addition, the Rowett Research Institute are investing in the new and powerful technologies of genomics and proteomics which allow the study of the relationship between the complex mixture of micronutrients in the human diet and their effects on gene expression in cells and tissues of the body. The Rowett Research Institute also has the unique capability to undertake nutritional research at the cellular level right up to studies with human volunteers, in its special Human Nutrition Unit.

Berry related research at the Rowett and collaborative work on salicylic acid.

1. It is now recognised that adequate intakes of antioxidant micronutrients are required to reduce the risk of developing clinical conditions such as heart disease and cancer. These are at unacceptably high levels in the Scots. However, the "Inverurie Study" which looked at the diets of adults in the north east of Scotland indicated that the majority of the population did not achieve appropriate amounts in their diets of important micronutrients such as vitamin E, vitamin C and carotenoids. The Rowett therefore started a programme to ascertain whether there were other types of foods containing novel antioxidants that the Scots could be persuaded to eat.
2. The Rowett initially became interested in polyphenols as it was thought they might be a substitute for vitamin E. Strawberries, raspberries and blackcurrants are a particularly rich source of polyphenols called anthocyanins which were found to be very potent antioxidants in the test-tube. Consequently, a collaborative project involving the Rowett, SCRI and University of Glasgow and funded by SEERAD was set up to explore the nutritional value of anthocyanins as antioxidants.
3. Although it was found that replenishing vitamin E deficient diets with anthocyanins reduced indices of DNA damage and lipid peroxidation in rats, the large amounts required to achieve this potentially protective effect could never be achieved with a normal diet and indeed at such high levels adverse effects can never be discounted. In addition, in a human study using cranberry juice as a source of anthocyanins, (funded by the Cranberry Institute in USA), no anthocyanins were found in the plasma or the urine even after consuming about a litre of juice/day for 2 weeks. These sorts of results suggest that anthocyanins may not be readily absorbed from the gut and so it is important to repeat this with Scottish fruit which will have different types of anthocyanins which may be more bioavailable.
4. The Cranberry juice experiment did show that soft fruit was an excellent source of vitamin C. In addition, salicylic acid levels in blood and urine went up after consumption of the cranberry juice. This compound is thought to have anti-inflammatory and anti-cancer properties and it is therefore important to know what the levels are in Scottish soft fruits (it was first discovered in strawberries in 1903!) Derek Stewart (SCRI) has therefore sent samples to John Paterson in Dumfries and Galloway Royal Infirmary for analysis.
5. Although anthocyanins may not be easily absorbed, it is still possible that they may have important protective effects in the gut. Garry also is set to work on a colon cancer project and the influences of bioactive compounds from soft fruit on colon cancer development.

Dr Donald McPhail – Rowett Research Institute

Electron Spin Resonance (ESR). Uses in relation to the antioxidant properties of fruit and its constituents.

Electron spin resonance (ESR) spectroscopy is a technique that can detect and characterise species containing unpaired electrons such as free radicals and paramagnetic transition metal ions. The method involves placement of a sample, contained within a quartz tube, in a microwave cavity situated between the poles of an electromagnet. The sample is irradiated then by scanning the magnetic field of the irradiated sample, a field strength occurs at which unpaired electrons with their spin-induced magnetic moments parallel to the field direction (low energy) can 'spin flip' by absorption of microwave energy, so that the magnetic moments are opposed to the external field (high energy). This absorption of energy is detected in the ESR experiment and results in a peak at a particular magnetic field strength. In addition to unpaired electron spin, many common nuclei (^1H , ^{14}N etc) also have spin. The interaction of nuclear spin with electron spin results in multi peak spectra. By determining the resonance positions and splitting patterns, structural information on the radical species can be obtained. ESR spectra can be highly-resolved with detection at the nanomolar level. It is free of interference from diamagnetic (non-radical) components and has the advantage of being able to work with turbid solutions, cell suspensions etc. which are problematic for optical detection systems.

The Rowett have developed two ESR methods that allow antioxidant activity of individual phytochemicals to be measured as well as the antioxidant capacity of the food matrix, such as berry juice or homogenate. For lipophilic compounds, galvinoxyl radical is used in organic solvents, and for hydrophilic species, Fremy's radical (potassium nitrosodisulphonate) is used in aqueous media. Both radicals have well-resolved and highly-characteristic spectra. In general, most workers using radical, or transition metal redox-based model systems (eg the TEAC, DPPH, FRAP assays) report results in terms of reaction stoichiometry, or an equivalent, once the reaction has gone to completion, however the rates of reaction are not known. Within a biological context, where a number of antioxidant species exist, and where protection of biomolecules is by competitive reaction of the antioxidant with a radical, reaction rates may be a prime determinant of efficacy. Consequently, researchers at the Rowett have utilised the fast response time of the ESR technique to develop stopped-flow methods to assess the rate constants of the reduction of our radical probes.

These methods have been used to look at the rates and stoichiometries of a large number of antioxidant phytochemicals found in fruits and vegetables. Focussing on the flavonoids, the second-order rate constants can vary between $ca\ 0 - 10^4\ \text{mol dm}^{-3}\ \text{s}^{-1}$, whilst the stoichiometries range from $ca\ 0 - 4$ molecules radical reduced / molecule of phenolic. Myricetin was found to be the most active of the group studied with respect to both parameters. However, the rates and stoichiometries did not always correlate. In particular, morin had only half of the stoichiometric activity of myricetin, but a similar rate. This highlights the importance of studying both parameters. Despite being the principal membrane protectant, vitamin E was ranked eighth in terms of radical scavenging ability and this demonstrates the danger of extrapolating data from simple chemical model systems. Tocopherol's efficacy is a likely consequence of the hydrophobic, farnesyl chain anchoring the active head group close to the membrane surface thus affording an optimum spatial distribution for radical interception.

The ESR technique has allowed good structure-activity relationships to be obtained for these polyphenols and to place this in the context of chemical resonance and electronic effects that pertain to the various molecules. Work at the Rowett has shown that hydroxyls of the A-ring system contribute little to the antioxidant activity and that potency synergistically increases by inclusion of a hydroxyl at the 3 position of the central C-ring along with the 4' position of the B-ring.

With respect to the radical quenching antioxidant assays it must be stressed that the results are not definitive; they are purely a research tool and have to be interpreted within the context of the system under investigation. Ultimately, these reactions are under thermodynamic control and consequently governed by the reduction potentials of the species involved. It is not surprising that different assays will give different results. The extent of oxidation and thus antioxidant activity will vary depending upon the reduction potential of the radical probe used

A factor, often ignored, and of considerable relevance to juice, is pH. The O-H groups will have different pK_a values, thus the extent of deprotonation will have significant impact. Proton loss results in the highest occupied orbital electrons being more diffuse and labile; there is less electrostatic attraction to the depleted positively charged core. Consequently, activity may rise for the anion of the parent molecule. Indeed, the reduction potential may rise to such an extent that electron transfer to molecular oxygen occurs; a paradoxical, pro-oxidant route *via* superoxide formation. Caution must therefore be exercised in the interpretation of such measurements.

The Rowett plan to use *in-situ* ESR electrolysis to obtain the exact thermodynamic potentials for a number of the redox probes currently in use.

The Fremy's radical technique has been used to assess global antioxidant activity for a large number of juices. Through knowledge of the reaction stoichiometry of individual phenolics, it has been possible to determine the contribution made to the overall capacity by individual components. The particular contribution of vitamin C to the activity of commercially available fruit juice was highlighted. In terms of commercial juice production, the impact of different recovery enzyme treatments on the antioxidant capacity of three blackcurrant cultivars was shown.

The wide detection range of ESR, its capability to work with turbid solutions, such as emulsions, and the availability of suitable hydrophilic and hydrophobic radical probes gives the technique the potential to provide valuable information on antioxidant phase partitioning attributes. From the classic octanol / water model, the LogP partitioning coefficient can be calculated from the antioxidant activities residing in aliquots taken from the two phases. Octanol extraction of fruit juice would give some understanding of the lipophilic / hydrophilic antioxidant balance. Furthermore, incorporation of radical into the aqueous or organic compartments of liposomes or emulsions would open up the possibility of assessing redox exchange at the interface.

Acknowledgements:

Garry G Duthie (RRI), Peter T Gardner (RRI), Bernard A Goodman (SCRI)

Prof Alan Crozier – University of Glasgow

Absorption and metabolism of dietary phenolics.

1. Fruits and vegetables contain minerals, fibre, vitamins and phytochemicals. It is the phytochemicals we are mainly concerned with today.
2. The flavonol content of different varieties of a fruit or vegetable can vary dramatically. Eg the cherry tomatoes *v* normal, red onions *v* white.
3. Phenolic constituents of soft fruit:
flavonols, catechins (flavan-3-ols), anthocyanidins, ellagitannins.
4. HPLC-MS MS analysis of Glen Ample raspberries can pick out quercetin molecules with various side chains, ellagitannins and kaempferol. This is a very sensitive method.
5. Ellagitannins are very large molecules which, if hydrolysed, release ellagic acid and gallic acid. Ellagic acid received a lot of attention in the late 1990s for its potential anti-cancer properties. Sanguin H6 (an ellagitannin) has a major contribution to antioxidant capacity in raspberries. Vasorelaxation activity falls within the ellagitannin fractions.
6. Freezing and storage treatments of Glen Ample raspberries:
Freshly picked berries, frozen berries, "shop" (4C for 3 days) and "home" (4C for 3 days then 18C for 24 hours) fruits were analysed for their ellagitannin, vitamin C and overall antioxidant capacities. The levels of

anthocyanins varied very little, while the ellagitannin levels went up slightly, vitamin C decreased slightly and the overall antioxidant capacity remained the same across all storage treatments.

7. In humans, there are only certain parameters that can be measured in the analysis of digestion and metabolism of phenolic compounds: the plasma, urine and faeces.

Can't look at tissues - must use animal models for this.

Glasgow University have a system whereby the ileal fluid can be collected from ileostomy volunteers, therefore can find which compounds and metabolites from the diet are entering the colon.

8. Metabolism of the flavonol quercetin-4-glucoside in rats:

Radio-labelled quercetin-4-glucoside was included in the diet of the rats. No quercetin-4-glucoside was left after 2 hours – had all been metabolised (19 separate metabolites identified) with the majority of the radioactivity remaining in the gut. (The rats were not overdosed).

9. Anthocyanins:

Are not metabolised like the flavonols. They are slightly bioavailable after lots of wine but after consumption of normal amounts, anthocyanins were not detected in plasma.

10. Concentrations of anthocyanins in plasma following ingestion of raspberries show individual variation and levels in plasma peak between 60 and 200 minutes.

11. Following consumption of raspberries, the ileal fluid was examined and 50% of anthocyanins remained intact. Where is the other 50%? Sequestered in the tissues or broken down to as yet undetected catabolites? In contrast to flavonols, there doesn't seem to be major metabolism of anthocyanins.

Could use rats and radiolabelled anthocyanins in an experiment similar to those carried out with flavonols to find what happens to the anthocyanins?

Questions raised:

What about the gut microflora – how will they affect absorption / metabolism of anthocyanins / phenolic compounds? Furthermore - everyone has different gut microflora populations.

Thoughts for any future work:

Synthesis of radiolabelled phenolics and flavonoids for experiments to determine their fate in model rat systems. Armed with this information carry out feeds with healthy humans and ileostomy volunteers with appropriate fruits and vegetables. To ascertain what compounds are absorbed into the circulatory system and which ones retain in the gastrointestinal tract. Investigate the effect of absorbed compounds on cardiovascular and related diseases and of the non-absorbed components on colon cancer.

Prof Jill Belch – University of Dundee

Cardiovascular Health and Nutrition

Clinicians are beginning to recognise the importance of nutrition in ill-health. For example, the British Medical Journal reported in 1998 that "over 70% of the world's ill health has a nutritional component".

The 5 year mortality rates for cardiovascular disease are higher than for breast and prostate cancers.

The white cell count is predictive of CVD with larger WCC conferring a higher risk (more so in men).

Levels of the adhesion molecule E-selectin are higher in people with angina than in controls, and higher still in those who have survived a heart attack.

There is a link between poor antioxidant intake and survival amongst those who have undergone vascular surgery.

A number of techniques exist for measuring markers of endothelial health and various studies have shown that many of these markers are predictive of disease and also that markers can be altered by dietary intake.

A small unpublished study showed that markers of endothelial damage were decreased when a meal was given with fruit. The numbers weren't big enough in this study to draw firm conclusions.

Examples of techniques used at the Vascular Diseases Research Unit headed by Prof Belch:

- a) White blood cell aggregation test (developed by Prof Belch's group).
- b) Measurement of various cell adhesion molecules.
- c) Platelet activation and aggregation tests.
- d) Laser doppler imaging. A non invasive technique for measuring blood flow in the skin:

Iontophoresis is a method for transporting drugs in solution across intact skin – convenient for producing a stimulus to the blood vessels in the skin.

Arterial compliance or *stiffness* can be measured by applanation tonometry. This records the peripheral artery blood pressure waveform and calculates aortic compliance.

Dundee Infant feeding study: 600 normal children who are now 12-14 years old. 50% had cholesterol higher than recommended levels and 20% had a diabetic sugar profile after hamburger, fries and milkshake meal. Endothelial function was shown to be poorer in these children.

Following cholesterol lowering treatment in patients, endothelial function was improved.

The importance of nutrition in health: mediated by fat, carbohydrate, antioxidants, vitamins, trace elements and micronutrients. The people involved in the work of the unit are: Dr M McLaren – thrombosis haemostasis, Dr F Khan – vascular imaging, Dr A Hill – oxidative stress.

Prof John Hayes – University of Dundee Cancer Chemoprevention

1. The *focus* of the work in the laboratory is the identification of molecular mechanisms by which cancer chemopreventive “blocking agents” protect against tumourigenesis. The central *hypothesis* being tested is that increased resistance to carcinogenesis can be afforded by xenobiotics that elicit an antioxidant adaptive response.

2. Antioxidants exert their effect by scavenging free radicals or by inducing expression of a battery of genes encoding antioxidant and detoxication proteins. The promoters of many of these genes contain a cis-acting element called an antioxidant response element (ARE) which is recognised by several basic-region leucine zipper (bZIP) transcription factors, such as Nrf1 and Nrf2.

3. Areas of interest are in:

- the characterisation of the nucleotide sequence that defines the ARE
- regulation of the ARE by transcription factors eg Nrf1 and Nrf2
- identification of human and mouse genes regulated by Nrf1 and Nrf2 through the ARE
- identification of agonists and antagonists of ARE-driven transcription (this includes phytochemicals such as polyphenolics, isothiocyanates, coumarin and organosulphur compounds)
- the biological consequence of stimulation of the ARE gene battery.

4. Reactive oxygen species (ROS) can damage lipids, DNA and proteins in the cell. Furthermore, carbonyls generated by the attack of ROS on lipids, sugars and protein can also damage DNA. Molecules such as glutathione, ascorbic acid and α -tocopherol can scavenge ROS, whereas drug-metabolising and antioxidant enzymes protect DNA from carbonyl-containing compounds generated by ROS. Although the body cannot store ascorbic acid or α -tocopherol, induction of drug-metabolising and antioxidant genes by cancer chemopreventive agents is important because the protective effects can last for a few days. Through gene induction, antioxidant capacity can be extended for a much greater length of time than can be achieved by ascorbic acid and α -tocopherol.

5. ARE-responsive genes can be induced by the isothiocyanate sulforaphane and tert-butylhydroquinone which, having entered the cell, generate a "chemical signal" (probably an oxidation signal) resulting in translocation of the Nrf2 bZIP transcription factor to the nucleus where, following heterodimerization with Maf, it activates ARE-driven gene expression and enhances antioxidant defences. Translocation of Nrf1 may also occur.

6. Examples of genes regulated through an ARE:

Antioxidant enzymes:

- cysteine transporter
- glutathione reductase
- superoxide dismutase
- thioredoxin reductase

NADPH regeneration enzymes:

- glucose-6-phosphate dehydrogenase
- 6-phosphogluconate dehydrogenase

Phase I drug-oxidation/reduction enzymes:

- aldehyde dehydrogenase 3
- microsomal epoxide hydrolase
- NADPH:quinone oxidoreductase

Phase II drug-conjugating enzymes and phase III drug efflux pumps:

- Glutathione-S-transferase (GST), α, μ, π classes
- UDP glucuronosyl transferase

7. Methods and expertise of the lab:

- Expression of ARE-regulated genes, biochemical assays, antibodies (protein) and RT-PCR (mRNA)
- Mouse nrf2 gene knockout mouse: whole animal and MEF nrf2^{+/+} and nrf2^{-/-} lines
- Knowledge of chemical agonists of the ARE pathway
- Human colon and rodent hepatic cell lines that can activate ARE driven gene expression
- Various ARE reporter gene assays: CAT and luciferase
- Expression constructs for Nrf1, Nrf2, small Maf, and Keap1.

Thoughts for any future work:

John is especially keen to collaborate with anyone who could help to measure free radicals in cell lines (maybe by ESR) to establish whether generation of such species precedes activation of ARE-driven genes following treatment with inducing agents such as sulforaphane, tert-butylhydroquinone, menadione, and BSO.

It would be interesting to assess whether polyphenolics in fruit can stimulate ARE-driven gene expression in some of the cell models we have. We could also look at XRE-driven gene expression, mediated (not by Nrf2 but) by the Ah receptor.

Someone should perform a microarray analysis of the changes in gene expression affected by polyphenolics. This would be conceptually useful as it would take berry juice beyond somewhat passive antioxidant function.

Discussion 2pm – 3:30pm

There was a brief discussion first of all when the group heard from the people who had not spoken in the morning:

Annie Anderson - Professor of Food Choice at the Centre for Public Health Nutrition Research, University of Dundee

Brian Williamson – SCRI

Stuart Gordon – SCRI

Wayne Powell – SCRI

Alistair Paterson – Strathclyde University

Jane Scott – University of Glasgow

Ronnie McNicol - Redevea

A summary of various funding opportunities was put forward.

Anna Whyte explained about the FSA Scotland funding situation: FSA Scotland does not put out calls for proposals but will consider any project that is deemed to have a Scottish theme. FSA Scotland is still developing it's ideas as to what constitutes a "Scottish theme" but will look at a short half page idea and decide whether it is worth expanding into a full proposal.

A summary of different funding bodies was presented including the EU framework 6, BBSRC, WCRF.

Vascular tests & feeding studies are obvious. Perhaps with different types of berries to assess the impact on markers of different levels of bioactive compounds. This could be applied to processed foods as well – but would need to work with the manufacturers to ensure consistency of quality.

T34 from EU Framework 6 programme was considered interesting. (Is an indicative topic for 2004.)

Can look at microarrays for general induction of gene expression.

Need good biological models, i.e. rat / mouse models for example the Big Blue Mouse.

Gut microflora impact could be important. Need to consult experts in this area – none present today. Colon health and protection from cancer is an important area.

It was agreed that details of activities / collaborations and projects should be given to Julie to put on the Berry Scotland Website.

Julie to collect together points raised at the meeting and circulate, with the possibility of further visits to scientists to get various funding proposals moving.