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Physiological Functions of Dietary Phytochemicals and Complex Carbohydrates

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ABSTRACTS

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CHARACTERISATION OF AN ORGANIC ANION TRANSPORT MECHANISM IN THE HUMAN COLON CARCINOMA CELL LINE HT29 CLONE 19A

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HT-29 is a commonly used human intestinal tumor cell line to study various aspects of the impact of nutritional components and metabolites on processes of colon carcinogenesis. Clone 19 has been subjected to differentiation by treatment with butyrate. In order to use this all clone for studies aimed at determining the effects of nutritional factors, it is necessary to characterize the cells' physiological functions. We have measured Fluo-3 efflux in cultured HT29 cl. 19A cells grown on cover slips to investigate a recently identified probenecid sensitive organic anion transporter in these human colon carcinoma cells (Pflügers Arch. 429: R56, 1995). Cells were loaded with Fluo-3 upon incubation with Fluo-3/AM and 2 mM probenecid. Intracellular Fluo-3 fluorescence of a group of 6-12 cells was measured at 37°C using epifluorescence microscopy and a photomultiplier. The decrease in intracellular fluorescence after 5 min incubation was calculated as the percentage of the total cellular fluorescence intensity at the start of each experiment. Fluo-3 efflux was significantly reduced in the presence of 2 mM probenecid (6.2 ± 3.7 % versus 22.8 ± 2.2 %, n=5-6, $P<0.01$). Fluo-3 efflux also tends to be lowered at 25°C (8.2 ± 1.3 versus 15.5 ± 3.7 %, n=5-6, $P<0.06$), but was not altered in the presence of 10 mM p-aminohippurate (18.0 ± 1.0 %, n=4), 10 mM pyruvate (22.0 ± 9.5 %, n=3), 10 mM α-ketoglutarate (16.0 ± 1.5 %, n=4), or 10 mM sulfate (14.5 ± 0.5 %, n=2). We therefore conclude that HT29 cl. 19A cells express a probenecid sensitive anion transport mechanism that is not stimulated after addition of p-aminohippurate, pyruvate, α-ketoglutarate or sulfate to the trans side.

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ESTABLISHMENT OF HUMAN COLON CELL MODEL WITH INTESTINAL PROPERTIES

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A new human colon cell line has been established by SV40 T antigen immortalization of human adult enterocytes in a serum free medium. The cells have an indefinite lifespan, a near diploid karyotype and were non tumorigenic in nude mice. The expression of cytokeratins (CK7,8,13) and the formation of tight junctions and desmosomes demonstrate their epithelial character. The differentiation and intestinal properties of the cells is confirmed by the expression of alkaline phosphatase and sucrose isomaltase after induction with TGF-β. Other functional properties include the expression of metabolic enzymes. The cells express cytochrome P450s (CYP450): CYP1A1, 2C, 2D6, 2E1, 3A4/5, with CYP3A at a similar level as in human colon tissue. Northern blot analysis of phase II and oxidant defence enzymes showed a strong expression of gluthatione-S-transferase π (GSTπ), quinone reductase (QR), epoxide hydrolase (EH), superoxide dismutase (SOD), glutathione peroxidase (Gpx) and catalase, indicating that the cells are competent in phase II metabolism and oxidative defence. Moreover, induction of HLA class II molecules (HLA-DR, -DP,-DQ) after exposure to IFNγ reflects the immunological competence of these cells. The interaction of HCEC cells with intestinal bacteria was demonstrated by adhesion assays.

The immortalized HCEC cells constitute a physiological model for studies in toxicology and carcinogenesis as well as a system for studying bacterial adhesion and immunological properties in the intestine.

VEGETABLE AND FRUIT CONSUMPTION AND CANCER RISK

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Epidemiological studies have shown that high intakes of fruit and vegetables are associated with reduced risk of cancer at several sites. The association is most marked for epithelial cancers, is apparently stronger for those of the alimentary and respiratory tracts, and somewhat weaker for hormone-related cancers. Scanty and inconsistent data are available for non-epithelial cancers. Raw and fresh vegetables showed the most consistent protection. We analyzed the relationship between cancer risk and frequency of consumption of green vegetables and fruit by using data from a series of case-control studies conducted in Northern Italy since 1983. The relative risks (RRs) for most common neoplasms ranged from 0.2 to 0.5 for the highest compared to the lowest tertile of vegetable intake. Protective effects were also observed against hormone-related neoplasms. Higher intakes of fruit were related to a reduced RR for cancers of the oral cavity and pharinx, esophagus, stomach or larynx, as well as of the urinary tract. No association was observed between fruit and vegetable consumption and non-epithelial neoplasms. For upper respiratory and digestive tract cancers, population attributable risks (ARs) for fresh vegetables and fruit intake ranged from 18% to 40% in men and from 15% to 30% in women; ARs for fresh vegetables and fruit intake, combined with tobacco and alcohol, exceeded 85% for men and 55% for women.

DETECTION OF OXIDATIVE DNA DAMAGE IN HUMAN INTERVENTION STUDIES

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Endogenous oxidation of bases in DNA is implicated in human carcinogenesis. Specifically, there are known epidemiological associations between high consumption of foods containing antioxidants, which are expected to decrease oxidative damage, and low levels of several cancers. However, intervention studies designed to test the hypothesis that antioxidant supplements should decrease cancer incidence have given equivocal and contradictory results. We have developed the comet assay (single cell gel electrophoresis) as a way of assessing levels of endogenous base oxidation in human lymphocytes, as well as the resistance of lymphocytes to in vitro oxidative damage - two useful biomarkers for assessing the effects of dietary antioxidants. At the end of a 20-week supplementation trial, with a cocktail of vitamins C and E and beta-carotene, we found a significant decrease in the frequency of oxidised pyrimidines in DNA, and an increased resistance to in vitro damage. Even a single, large dose of vitamin C, vitamin E or beta-carotene given before blood sampling decreases the yield of oxidative damage in the lymphocytes. The damage that does occur to DNA is potentially mutagenic, but most of it is removed by DNA repair before DNA replication (and fixation of mutations) occurs. The comet assay can be used to follow the kinetics of removal of strand breaks and oxidised bases resulting from oxidative attack on DNA. However, freshly isolated, unstimulated lymphocytes seem to recover very slowly compared with cultured cell lines. We have evidence that the increased oxygen tension encountered by lymphocytes in vitro results in continuing oxidative damage that confounds the repair process. There are inter-individual differences in the ability of dietary antioxidant supplements to enhance the recovery from damage, and these differences reflect the changes seen in the levels of antioxidants in the plasma. Whether micro-nutrients actually stimulate DNA repair, or simply decrease damage, is not clear. To answer this question, we are developing a different approach, measuring DNA repair enzyme activities in lymphocyte cell-free extract, again using the comet assay.

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EFFECT OF DIFFERENT STARCHES ON THE HUMAN GUT MICROFLORA ACTIVITY IN *IN VITRO* CULTURE

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Several studies have shown a protective effect of dietary starch on the development of colonic cancer. Such starches, introduced with diet, escape digestion by the small bowel and reach colon, where they are broken down by microflora to yield a variety of the factors that protect the mucosa (short chain fatty acids - SCFA - chiefly represented by acetic, propionic and butyric acids). On the other hand the production of SCFA will lead to a decrease in pH, resulting in an inhibition of the enzymatic transformation of primary into secondary bile acids (BA). Secondary bile acids are co-mutagenic and cytotoxic, resulting in an increased cell proliferation. However, different starches seem to have different effects on colon physiology and on the metabolic activities of the intestinal flora (1,2,3).

The aim of our study was to compare the fermentation products of different starches (range 6 - 70% amylose), using both human faecal microflora and several bacteria species isolated from the same faecal flora. The experiments were conducted using a semi-continuous culture model of the human faecal microflora which has been shown to mimic both the bacterial composition and the metabolism of the *in vivo* microflora.

We characterized the faecal flora, and measured functional changes in flora by determining metabolic products (SCFA and secondary BA) and enzyme activities.

Preliminary results revealed a variation in the capacity of the different types of starches tested in modulating the microflora and the metabolic activities. Surveys on faecal isolates for the ability to ferment starches have shown that some bacterial species (*Bacteroides: vulgatus, ovatus; Clostridium: bifermentans, beijerinkii, butyricum and Propionibacterium acnes*) were the most versatile utilizers of normal maize starch. The fermentability was proportional to the production of total SCFA and revealed different levels of production of both total SCFA and several acids from various tested starches.

1) Cassidy,A.,Bingham,S.A. and Cummings.J.H. (1994). *British Journal of Cancer* **69**, 119-125.

2) Englyst,H.N. and Cummings, J.H. (1986). *American Journal of Clinical Nutrition* **44**,42-50.

3) Mallett, A.K., Bearne, C.A. and Rowland, I.R. (1989). *Journal of Applied Bacteriology* **66**,433-437.

EFFECTS OF PROCESSING ON THE LEVEL AND BIOACTIVITY OF ANTIOXIDANTS IN FOOD PRODUCTS

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Intake of several antioxidants has been associated with lower incidence in various aging deseases. It is important to know what the effects of processing steps are on the level and activity of these compounds in (processed) foods. With this information more accurate figures can be given for epidemiological work and also product development can be directed to consumer foods with higher content of antioxidants. As examples antioxidants in apple and tea are studied. Flavonoid content in applejuice is only 5-10% that of the apples used to produce the juice. The conventional process of making juice from apples has been investigated for the losses of flavonoids. Alternative processes have been shown to be possible in order to significantly improve the level of flavonoids in the final product. Catechin content in tea varies widely among types and brands. This has marked effect on the antioxidant activity of tea. The contribution of catechins to the total antioxidant activity of tea infusions has been studied as well as some of the effects of the infusion process on the final level of antioxidant activity.

EFFECT OF DIFFERENT CARBOHYDRATES ON INTESTINAL FUNCTION AND CHEMICAL CARCINOGENESIS

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We have studied whether starches (maize starch) or simple sugars (glucose fructose and sucrose) modify colon function and the process of chemical carcinogenesis at the level of the colon. Feeding rats a diet in which carbohydrates are represented by starch, modifies ways colon function differently than in animals fed sucrose. After a period of 30 d in the animals fed starch, colon pH decreases and the levels of short chain fatty acids and the ratio butyrate/acetate in the colon content increase. This is associated with a higher cellular proliferation in the sucrose-fed animals, a known risk factor for the induction of colon cancer. When animals are treated with colon specific carcinogens like 2,3-dimethylhydrazine (DMH) or azoxymethane (AOM), preneoplastic lesions like aberrant crypt foci (ACF), adenomas and carcinomas are induced. Animals fed starch have a slower growth of ACF, and a smaller number of intestinal adenomas compared to the sucrose fed rats.

The type of carbohydrate consumed in the diet is important, as is their administration schedule and route. In fact, when administering simple carbohydrates, such as sucrose or fructose, in the form of a bolus on an empty stomach, a burst of proliferation is observed in the colon mucosa and an increased expression of ACF after AOM administration. We tested the additional effect of buluses of sucrose on intestinal carcinogenesis by administering sucrose as a bolus in a chronic carcinogenesis experiments after induction with AOM. In these experiments, carried out with Fisher 344 rats, we confirmed a protective effect of starch vs. sucrose or glucose on the induction of intestinal adenomas, but not against the induction of adenocarcinomas. However, no further effect of the administration of sucrose as a bolus was observed vs. sucrose or glucose administered continuously with the diet. All these data seem to indicate a modest, but significant slowing effect of complex carbohydrates vs. sucrose on the progression of colon neoplasia, which may have some relevance for human nutrition and cancer. These effects are by no means dramatic and do not seem to be associated with the way of administering carbohydrates or with their glycemic index.

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ANALYSIS OF COMPLEX OLIGOSACCHARIDES FROM HUMAN MILK BY MATRIX-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY (MALDI-MS) AND HIGH-PH ANION-EXCHANGE CHROMATOGRAPHY (HPAEC)

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Human milk contains 1% (w/v) oligosaccharides (without lactose). Up to now, neither the biological functions of the carbohydrates nor most of the structures of the oligosaccharides have been revealed. Two main functions have been proposed:

1) Oligosaccharides are part of an anti-infective system of new-born humans and work as soluble receptor analogs of glycan structures of cell-surfaces of the gastro-intestinal system.

2) Oligosaccharides promote the growth of certain indigenous bacteria (e.g. Bifidus spec.) while suppressing undesirable bacteria .

Studies indicate that either each of the stragegies or a combination of both prevent infections in breast-fed infants. With MALDI-MS neutral oligosaccharides of up to a molecular weight of 8.000 Da have been found in GPC fractions. With HPAEC some of the known characterized neutral and acidic structures have been identified and quantified. Thus a combination of both techniques will be a powerful tool for characterizing distinct oligosaccharides used in in-vitro or in-vivo assays which are aimed at revealing the biological functions of the complex carbo-hydrates.

Stahl, B., Thurl, S., Zeng, J., Karas, M., Hillenkamp, F., Steup, M., and Sawatzki, G. Anal Biochem. 1994, 223, 218-226

Thurl, S., Müller-Werner, B., and Sawatzki, G., Anal. Biochem. 1996, 235, 202-206

MECHANISTIC INVESTIGATION OF POTENTIAL CANCER CHEMOPREVENTIVE AGENTS FROM PLANTS

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Cancer chemoprevention is defined as the use of chemicals or dietary compounds to block, inhibit or reverse the development of cancer in normal or preneoplastic tissue (1). Modulation of drug-detoxication enzymes is one mechanism to block carcinogenesis at the initiation stage. As a result, our program for the detection of novel cancer chemopreventive agents, brassinin, an indole-based dithiocarbamate found in cruciferous vegetables like Chinese cabbage, and brassinin derivatives were shown to induce drug-metabolizing enzymes at the transcriptional level and to possess significant chemopreventive activity (2).

Rotenoids, related to the plant-derived insecticide rotenone, were identified as a novel class of anti-tumor promoters (3).

As an example, deguelin was found to mediate potent chemopreventive activity through transcriptional regulation of phorbol ester-induced ornithine decarboxylase (ODC) activity.

(1) Sporn, M.B., and Newton D.L. Fed. Proc. **38**, 2528-2534, 1979.

(2) R.G. Mehta et al. Carcinogenesis **16** (2), 399-404, 1995.

(3) C. Gerhäuser et al. Nature Med. **1** (3), 260-266, 1995.

DETECTION METHODS FOR FOODS DERIVED FROM GENETIC ENGINEERING

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During the debate about the use of genetic engineering in food production there was and still is an intensive discussion about detection and labelling of foods derived from genetic engineering. Detection methods for these types of foods could be based on the newly introduced genetic information. Because of its high sensitivity, its specificity and rapidity, the polymerase chain reaction (PCR) will be the method of choice for this purpose.

With PCR there is a possibility to amplify specifically very little amounts of DNA with primers corresponding to the gene of interest. If no inhibitory factors interfere with the PCR-reaction, 10 to 20 target molecules are sufficient to allow successful amplification and subsequent identification. However, when applied to food samples the PCR can be inhibited or its sensitivity can be severely reduced. Using complex food matrices as a DNA source, detection limits about 100-times less were achieved by agarose gel electrophoresis and about 15-times less by specific DNA-hybridization. This could be attributed to difficulties in extracting DNA from complex food matrices and reduction to a sensitivity of the PCR by several food compounds. The difficulties in extracting DNA from food matrices may be due to binding of DNA and microorganisms to food ingredients, thus interfering with the release of nucleic acid. The inhibitory effect on the PCR might be linked to the precipitation or denaturation of the DNA as well as to the denaturation or inhibition of the DNA-polymerase.

Requirements for the PCR are the availabilty of adequate intact recombinant de-oxyribonucleic acid (rDNA), the knowledge of the genetic modification (necessary to design specific PCR-primers) and that this genetic modification is not exclusively due to genes of the own species (self-cloning). Intact DNA is available, if the food is the genetically engineered organism, itself or if the food contains genetically engineered organisms. In food containing isolated or processed products from genetically engineered organisms, but not the genetically engineered organism itself a clear detection will be possible in exceptional cases only! e.g. if there is still rDNA of sufficient fragment length or if an ingredient normally not found in this organism is produced. For example in pizza tomatoes, peeled tomatoes, french fries, fried potatoes and potato crispes, DNA suitable for PCR was found. Therefore it should be possible to detect that

these foods were derived from genetic engineering. Such a detection is impossible in beer, tomato soup, potato flour, mashed potatoes or soya been oil, since PCR-analysis gave no indication of the presence of DNA in these products. In principle, the used method is able to detect specifically little amounts of DNA in such products, which was shown by adding *Escherichia coli* DNA. For isolated products which are identical to conventionally produced ones such a detection is impossible to manage.

STRATEGIC FOOD CROPS: EMPLOYMENT OF PLANT BREEDING TO ENHANCE CONTENTS OF PHYTOCHEMICALS AND COMPLEX CARBOHYDRATES.

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The paper will review some examples where levels of secondary metabolites and complex carbohydrates have been modified. It will concentrate on fruit and vegetables as much as possible, but will include cereals for a few examples. It will also focus on examples where the levels of "beneficial" compounds has been increased; there are also many examples where the levels of toxic or harmful secondary metabolites and complex carbohydrates have been reduced. Historically the main target in breeding for nutritional quality has been protein content and quality, with a rather stronger focus on animal rather than human nutrition. There have been examples such as opaque-2 high-lysine maize where the target has been for enhanced human nutritional quality, though the level of success in terms of widespread use of this type of maize has been limited. Fat content has also been increased, but this has generally been targeted at crops grown for oil extraction rather than for direct consumption of the whole grain. More recently, with the increasing understanding of the relationship between fruit and vegetable consumption and the incidence of degenerative diseases, there has been a change in interest from simply overcoming nutritional deficiencies to enhancing levels of the secondary metabolites and complex carbohydrates that are implicated in reducing the incidence of such diseases.

The genetics of variation in carotenoids has been extensively studied in several crops, notably carrots and tomatoes. It has been possible to develop carrot lines containing up to 500 ppm total carotenoids as compared to 60-130 ppm found in normal orange carrots (Simon et al , 1989). Tomato varieties have been bred to contain 200 ppm lycopene as compared with the normal 50-60 ppm (Mir, 1996) and orange tomatoes have been selected with up to 80 ppm beta-carotene as compared with the normal 5-8 ppm (Kœhler et al., 1947). There have been

cases where crop varieties with high levels of particular carotenoids have failed to do poor consumer acceptability.

The high beta-carotene tomatoes described above were rejected due to their orange colour (Tomes & Quackenbush, 1958) and yellow maize, which has a much higher level of beta-carotene, is rejected in favour of white maize by consumers in many countries, even those which have a severe incidence of vitamin A deficiency.

The genetics of variation in tocopherol has been less extensively studied with no information available on breeding for enhanced tocopherols in vegetables and fruit. Skoric et al. (1996) have developed sunflower lines where the normal 95-100% alpha-tocopherol is 50% replaced by beta-tocopherol or 95-100% replaced by gamma-tocopherol. These new types will have higher antioxidant activity, but will be relatively deficient in vitamin E activity.

Breeding and selection for enhanced vitamin C levels has been a major target in several fruit and vegetable crop species. For example, Wang et al. (1994) have used interspecific hybridisation to breed high-ascorbate kiwifruit, while there is a long history of breeding and selecting blackcurrant for high ascorbate content (Astakhov, 1994, SHRI., 1979). Kelly (1954) reports a threefold increase in ascorbate content of potatoes after two generations of selection. The -hp (high pigment) and -dg (dark green) mutants of tomatoes show enhanced ascorbate levels (Jarret et al, 1984) and the progenies from interspecific hybridisation with the wild species Lycopersicon pimpinellifolium have shown a high heritability for enhanced ascorbate content (Pospisilova & Betlach, 1973). Bhagyalakshmi et. al. (1990) have reported a high heritability for ascorbate content in peppers.

Isothiocycanates derived from hydrolysis by myrosinase of certain glucosinolates from cruciferous plants have been strongly implicated as having anticarcinogenic effects due to Phase 2 enzyme induction (Zhang & Talalay, 1994). The precursor glucosinolates which have been most strongly associated with Phase 2 enzyme induction are glucoraphanin (Zhang et al, 1994) and glucoiberin (Tawfiq et al, 1995). Glucoraphanin is the dominant glucosinolate in broccoli and glucoiberin dominates in Brassica rupestris (Mithen et al, 1987).

The genetics of the biosynthesis of glucosinolates has been extensively investigated in Arabidopsis and Brassica species (Mithen et al, 1995), and it should be possible byconventional means to breed vegetable brassicas containing the desired glucosinolates.There has been little work on conventional breeding specifically to modify levels of complex carbohydrates in fruits and vegetables, though breeding for alterations in texture is likely to have altered the

proportions or amounts of different complex carbohydrates. Complex carbohydrates have been extensively studied in cereals; for example, beta-glucan, which is found in oats and barley, has been implicated in the reduction of blood cholesterol levels
(Newman & Newman, 1991). The genetics of variation in beta-glucan content in barley is well understood (Greenberg, 1977) and barley has been successfully bred for reduced beta-glucan content to improve malting quality. There is no reason why it should not be bred equally successfully for high beta-glucan content for health benefits.

Astakhov, AI Sadovodstvo i Vinogradarstvo. 1993, No. 3, 19 21. Bhagyalakshmi, PV; Ravishankar, C; Subrahmanyam, D; Babu, VG South Indian Horticulture.1990, 38: 1, 15 17 Greenberg, DC, 1977 Theor. Appl. Genet 50 p41-46 Jarret, RL; Sayama, H; Tigchelaar, EC Journal of the American Society for Horticultural Science. 1984, 109: 6, 873 878 Kelly, WC In: The National Potato Breeding Program. FJ Stevenson (ed.) 130-134 Kohler, GW; Lincoln, RE; Porter, JW; Zscheile, FP; Caldwell, RM; Harper, RH; Silver, W, 1947 Bot. Gaz. 109: 219-225 Nir, Z, 1996 Pers. Comm Mithen, RF; Lewis, BG; Heaney, RK; Fenwick, GR, 1987 Phytochemistry 26:7 p 1969-1973 Mithen, R; Clarke, J; Lister, C; Dean, C, 1995 Heredity 74 p 210-215 Newman, RK; Newman, CW, 1991 Cereal Foods World 36:9 p800-805 Pospisilova, J; Betlach, J Rostlinna Vyroba. 1973, 19: 1, 89 96 SHRI, 1979 Scottish Horticultural Research Institute: 25th annual report for the year 1978. 1979, 145pp. Dundee, UK. Simon, PW; Wolff, XY; Peterson, CE; Kammerlohr, DS; Rubatzky, VE; Strandberg, JO; Bassett, MJ; White, JM HortScience. 1989, 24: 1, 174 Skoric, D; Demurin, Y; Jocic, S Proceedings of 14th International Sunflower Conference, Beijing/Shenyang, China 12-20 June 1996 Tawfiq, N; Heaney, RK; Plumb, JA; Musk, SRR; Williamson, G, 1995 Carcinogenesis 16:5 p1191-1194 Tomes, ML; Quackenbush, FW, 1958 Econ. Bot. 12: 256-260 Wang, SM; Huang, RH; Wu, XW; Kang, N Journal of Fruit Science. 1994, 11: 1, 23 26 Zhang, Y; Talalay, P., 1994 Cancer Research (Suppl) 54, 1976s-1981s Zhang, Y; Kensler, TW, Cho, C-G; Posner, GH; Talalay, P., 1994 Proc. Nat Acad. Sci. USA 91p 3147-3150

FLAVONOLS AND ANTHOCYANINS OF SELECTED VEGETABLES - STATUS, ANTIOXIDATIVE AND ANTIGENOTOXIC PROPERTIES

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Flavonoids as an integral part of the human diet are reported to be absorbable from the intestinal tract as glycosides as well as their microbially released aglycons and metabolites. We investigated the natural composition of flavonoids in selected vegetables and determined the antioxidative and antigenotoxic properties of some glycosides and the corresponding aglycons. French beans contain the 3-0-rutinosides and 3-0-glucuronides of quercetin (Q) and kaempferol (K), while 3,4'-0-diglucoside and 4'-0-glucoside were identified as components of onions. Red onion additionally synthesizes anthocyanins that were characterized as glucosides of cyanidin (C). Their structure is still under investigation.

The antioxidative activities of the flavonoids were higher in several cases as compared to trolox. The aglycons Q and C showed antigenotoxic properties *in vitro*, but K and several glycosides enhanced DNA damage caused by reactive oxygen species.

To evaluate the role of such polyphenols in the prevention of degenerative diseases our future investigations have to comprise bioavailability of flavonoids and their metabolites and future biologically relevant systems to clarify their mechanisms of action.

DETERMINATION OF FLAVONOIDS IN PLANT FOODS AND BIOLOGICAL FLUIDS

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Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different flavonoids have been described. Flavonoids have a variety of biological effects in numerous mammalian cell systems, in vitro as well in vivo. Recently much attention has been paid to their anti-oxidant properties and to their inhibitory role in various stages of tumour development in animal studies.

Analytical research of flavonoids was mainly aimed at identification and not at quantification. Consequently, no data for epidemiological investigation of flavonoid intake and chronic diseases were available. Flavonoids in foods are mostly linked to sugars, the so-called glycosides. As one parent compound or aglycone, e.g. quercetin, may be linked to a number of different sugars, quantification in foods will be complex. Hydrolysis of the glycosides and subsequent determination of the parent aglycones, will simplify this task. Following this approach we developed and validated an HPLC-method, and determined the flavonol and flavone content of vegetables, fruits and beverages commonly consumed. Subsequent epidemiological evaluation showed that the intakeof flavonols and flavones was inversely associated with coronary heart disease in both a prospective cohort study, and in a cross-cultural study. However, no relation with cancer risk could be established. The antioxidant properties of flavonoids offer a plausible explanation for the effect found. However, the extent of absorption of flavonoids is an important unsolved problem in judging their potential role in the prevention of coronary heart disease. To be able to study absorption of flavonols in man, we developed a postcolumn derivatization with aluminum for HPLC with fluorescence detection. Ultra Violet detection did not meet the requirements of sensitivity and specificity in biological fluids. Variables governing postcolumn chelation, such as water content, buffer and organic modifier of the eluent, concentration of Al3+ and presence of acetic acid in the postcolumn reagent, and temperature, were studied and optimized. Of the flavonoids, only flavonols that contain a free 3-hydroxyl and 4-keto oxygen binding site form fluorescent complexes with Al3+. The method has a detection limit of 0.15 ng/mL for querce-

tin, 0.05 ng/mL

for kaempferol, 0.45 ng/mL for myricetin, and 0.05 mg/mL for isorhamnetin, thus improving detectability of quercetin 300 fold as compared to that possible with UV detection. This extremely sensitive method was used to determine the absorption and disposition kinetics of flavonols after consumption of a normal diet.

PROTECTION OF RESISTANT STARCHES ON THE DEVELOPMENT OF INTESTINAL ADENOMAS IN THE APC^{MIN} MOUSE MODEL

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Many publications suggested that the content of dietary fiber influences the incidence of colon cancer. Therefore we investigated the effects of different sutructural types of resistent starches (RS) on normal and Apc^{MIN} mice. The Apc^{MIN} mouse model is characterized by spontaneous intestinal neopplasia caused by an autosomal dominant heterozygous nonsense mutation, converting codon 850 in the Apc gene from a leucine (TTG) to amber (TAG). The advantages of this Min mouse in comparison to many other carcinogen-induced tumor models are the clearly defined relevant genetic lesion, the analogy to the genetic predisposition for human colon cancer of patients with familiar adenomatous polyposis (FAP) and the possibility to study nutrive and drug effects under in vivo conditions.

Comparative studies hae been done with three structurally different types of RS over a period of 60 days. Test parameters have been clinical manifestation of the disease, the number and size of intestinal adenomas, the weight of spleen, the rate of bile acid dehydroxylation and the antioxidative capacity of blood plasma. Disturbances in the mucosal integrity of intestinal epithelial cells and balance of growth and differentiation as well as the state of tumor development have been assessed by normal and immunofluorenscence microscopy. Furthermore, the effects of RS on strain composition of microflora and the formation of short chain fatty acids have been analyzed. From the results it can be concluded that the protection of RS types against intestinal adenomas increases with the ability of microbes to absorb and degrade RS and the proportion of butyrate formers among the microbial strains. But also, well fermentable RS alone can only delay but not totally suppress the intestinal tumor development in Min mice.

SELENIUM STATUS OF SELECTED FOOD AND DIETARY HABITS OF CANCER PATIENTS

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The aim of this paper was to find out if there are some differences in dietary practices between cancer patients and healthy controls in different communities as well as to correlate nutrition and selenium serum levels. The investigation was conducted in two Belgrade communities, one rural and the other central with two groups of cancer patients (N=74) and healthy controls (N=54). Dietary habits were assessed by food frequency questionaires. The mean value for wheat was lower than average in samples from other parts of Serbia (20.5 +/ 12.4). Corn is extremely poor in selenium, with an average of 3.3, and garlic also, 3.3 μg/kg. Consumption frequency was calculated for 44 food items commonly used in our country. No significant differences were found for most of the matching controls and cancer cases, except for sugar ($p=0.005$) and fruit ($p=0.0083$). When Comparing the dietary practices in different communities, statistical significancy was found for the consumption frequency of different types of bread. All very rarely consume raw vegetables and salads - only 8 of 94 eat this type of bread every day. When the correlation analyses was done, no significant association was found between consumption frequency and serum selenium level.

DETERMINATION OF PLANT POLYPHENOLS IN FRUIT JUICES BY HPLC-UV AND LC-MS DETECTION

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The large group of plant polyphenols is known as natural antioxidants, and attracts attention because of their potential anticarcinogenic and antiatherogenic properties. The flavonoids are of major interest, as they are relatively prevalent in foods.

In order to estimate the daily intake of flavonoids in the diet, we initiated an investigation to identify and quantify the flavonoids in Danish foods and beverages. Flavonoids were identified and quantified (as aglycons) by HPLC-UV and mass spectrometry. Initially, we selected the five flavonoids most likely to be found in common foodstuffs, i.e. apigenin, kaempferol, luteolin, myricetin, and quercetin. During the investigation we have found other related flavonoids in considerable amounts, e.g. hesperitin and naringenin.

We have analysed various types of fruit juices: apple, orange, and black currant for the contents of flavonoids, and the results will be presented and compared to the analyses of the corresponding fruits.

INTRACELLULAR SIGNALS INVOLVED IN THE DCA-SCFA- AND FECAL WATER-INDUCED APOPTOSIS IN HUMAN COLON TUMOR CELL LINE, HT-29

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Bile acids (BA) and short chain fatty acids (SCFA), both components present in the feces and in direct contact with the colorectal epithelium, have been implicated in the etiology of colorectal cancer. BA, secreted in the intestine after a high fat intake, may act as a tumor promoters (1) while (SCFA), derived from the fermentation of fibers by the intestinal bacterial, are able to reduce cell proliferation and are considered as tumor-protective agents (2). Recently, we (3) and others (4) have demonstrated that both these compounds and their mixtures can induce the apoptotic cell death in human carcinoma cell lines, HT29 and CaCo2. The bile acid, deoxycolic acid (DCA) evoked a high level of apoptosis while SCFA had a weaker effect requiring higher concentrations and longer incubation times. It was found that neither protein kinase C, protein tyrosine kinase activities, nor *de novo* protein synthesis were critical events for either DCA- or SCFA-induced effects. The mechanisms of apoptosis induction were further investigated by studying the involvement of intracellular Ca^{2+}concentrations. Coincubation of the cells with the intracellularCa^{2+} chelator BAPTA/AM resulted in the inhibition of apoptosis induced by DCA while no effect was observed when apoptosis was triggered by SCFA. Accordingly, DCA induced a rapid increment of intracellular Ca^{2+} concentration as evidenced by the fluorescent probe FURA-2, whereas SCFA did not affect the cation homeostasis. Since the activity of phospholipase C could mediate the rise of intracellular Ca^{2+} by the production of inositol-triphosphate which promotes the release of Ca^{2+} from the intracellular stores, the effect of specific inhibitors of this enzyme was assayed as possible apoptosis inhibitors. Finally, we also evaluated the effect of fecal water obtained from three groups of rats fed with different diet composition on the apoptosis Ca^{2+} of HT29 cells, together with the role that Ca^{2+} would play on this effect.

1) Bruce, W.R., 1987, Cancer Res. 47: 4237-4242

2) McJntyre, A., Gibson, P.R. and Young, G.P. , 1993, Gut 34: 386-391

3) Marchetti, C., Migliorati, G., Riccardi, C., Scardazza, F., Mastrandrea, V. And Morozzi, G, 1996, Second International Congress on Food and Cancer Prevention, The Netherlands, Abstracts 193

4) Hague, A., Elder, D.J.E., Hichs, D.J. and Paraskeva, C., 1995, Int.J. Cancer 60: 400-406

METHODS TO EVALUATE REACTIVE OXYGEN SPECIES IN THE FAECAL STREAM

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The hypoxanthine/xanthine oxidase enzyme system is known to produce the superoxide ion and hydrogen peroxide during the hydroxylation of hypoxanthine via xanthine to uric acid. When chelated iron is included in this system, superoxide reduces iron III to iron II and the Fe-II-chelate further reacts with hydrogen peroxide to form the highly reactive hydroxyl radical. Because reactive oygen species, the hydroxyl radical especially are implicated in the carcinogenic process a high performance liquid chromatography method utilising the ion-pair reagent tetrabutylammonium hydroxide and the use of salicylic acid as an aromatic probe for quantification of hydroxyl radical formation was set up. In the hypoxanthine/xanthine oxidase system the major product of hydroxyl radical attack on salicylic acid is 2,5-dihydroxy benzoic acid. Comparison of phosphate and Tris buffer systems shows that the former is far suprior (90 %) in supporting Fenton chemistry because Tris also scavenges the hydroxyl radical. That the hydroxyl radical is involved in the hydroxylation of salicylic acid in these systems was demonstrated by the potency of dimethyl sulphoxide, butanol and ethanol to act as scavengers and dose-dependent inhibition by catalase and superoxide dismutase. Phytic acid, which is considered to be an important protective dietary constituent against colorectal cancer, inhibited hydroxylation of salicylic acid at a concentration one order of magnitude lower than the classical scavengers but was only effective in the absence of EDTA. The method has been applied to the study of free radical generation in faeces and preliminary results indicate that components of the faecal stream are able to produce reactive oxygen species in abundance.

ANTICARCINOGENIC PROPERTIES OF ROSEMARY STUDIED IN HUMAN CELL CULTURE MODELS.

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Natural polyphenolic compounds are found in many common foods, examples being tea and rosemary. These compounds are very potent antioxidants and have applications in food stabilization due to their ability to protect against peroxidation of oxygen sensitive foods. More recently, it has been shown in animal models that rosemary or tea polyphenols also have anticarcinogenic properties, being effective inhibitors of both tumour initiation and tumour promotion. It is therefore of interest to investigate the potential impact of these compounds on human health and to understand the mechanisms by which they act. We have used human liver and bronchial cell culture models to investigate the molecular mechanisms involved in the chemopreventive action of natural polyphenols. Rosemary extract, or its active components, carnosol or carnosic acid proved to be very potent inhibitors of DNA adduct formation induced either by the liver carcinogen, aflatoxin B1 or by the lung carcinogen, benzo(a)pyrene. Epigallocatechingallate, the most active antioxidant from green tea, was a less effective inhibitor of DNA adduct formation. Inhibition of the genotoxic effects of these carcinogens may occur by at least two pathways: (i) inhibition of the metabolic activation step catalysed by the phase I cytochrome P450 enzymes, (ii) induction of the detoxification pathway catalysed by the phase II enzymes such as glutathione S-transferase. We found that carnosol had potent effects on each of these pathways. These results in human cell models give some insight into the different mechanisms involved in the chemopreventive action of natural antioxidants in relation to phase I and phase II metabolism.

DETERMINATION OF TOTAL OXALIC ACID ONTENT IN THE RAW AND COOKED SPINACH

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Raw and cooked spinach were analyzed for total oxalic acid content using the precipitation method and this was compared to the enzymatic method. The enzymatic method was used as a reference method, to enable reliable estimation of oxalic acid exposure. In addition, dry matter and total organic acid contents were measured.

Oxalic acid levels determined by the precipitation method ranged from 3.61 g/100 g of dry weight in raw spinach to 2.69 g/100 g of dry weight in cooked spinach. These levels were significantly higher ($p < 0.05$) than those determined by the enzymatic method, which ranged from 3.01 g/100 g of dry weight in raw spinach to 1.93 g/100 g of dry weight in cooked spinach. Cooking of spinach involves a much lower loss of total oxalate content than expected.

The data were analyzed by analysis of variance to determine the functional relationship between the two different methods. The regression line equation and the relative error of the precipitation method were calculated.

The enzymatic method shows promise for the faster determination of oxalic acid in vegetables.

HUMAN INTERVENTION STUDIES WITH BRUSSELS SPROUTS: RESPONSIVENESS OF BIOMARKERS INDICATIVE OF CANCER PREVENTION.

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Many studies have linked diets rich in fruits and vegetables to prevention of human cancer. Mechanisms to explain this include antioxidant protection of biomolecules, but also induction of detoxifying enzyme systems by breakdown products of glucosinolates in cruciferous vegetables such as Brussels sprouts. We have performed studies in human volunteers using biomarkers to investigate whether these mechanisms indeed occur in humans, and could thus contribute to cancer prevention.
In the first study, 10 male volunteers consumed a diet free of cruciferous vegetables for three weeks. Five volunteers continued on a diet with 300 grams of Brussels sprouts per day. The other five volunteers continued with 300 grams glucosinolate-free vegetables per day. Levels of 8-oxo-7, 8-dihydro2'-deoxyguanosine (8-oxodG) in urine were decreased by 28% after the sprouts treatment, while plasma levels of α-class glutathione S-transferase were increased by 40%.
In a second study, in collaboration with the University of Nijmegen, we used a cross-over design with again 300 grams of Burssels sprouts or 300 grams of glucosinolate-free vegetables for one week. 5 male and 5 female volunteers participated. Plasma GST-α increased 1.5 fold during sprouts consumption in males, but not in females, while plasma GST-π remained unchanged. Biopsies demonstrated an increase in rectal GST-α (30%) and π (15%) but not in ducdenal GST's.
Our studies suggest that both enhanced antioxidant protection as well as increased detoxification enzyme levels may contribute to cancer prevention by cruciferous vegetables.

MECHANISMS OF PROTECTIVE EFFECTS BY COMPLEX CARBOHYDRATES, AND INTESTINAL BACTERIA DURING COLON CARCINOGENESIS.

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Complex carbohydrates may lead to an increase of lactic acid-producing bacteria (LAB) in the gut. It is expected that LAB may contribute to colon cancer prevention. We have studied the ability of LAB to prevent genotoxicity in colon cells (1,2). Using the Comet assay, our studies in rats *in vivo* have shown that *L. casei, L gasseri, L. acidophilus, L confusus, B. breve* and *B. longum, S. thermophilus* and *L. delbruckei* ssp. *bulgaricus* prevent DNA damage induced by N-Methyl-N-nitro-N-nitrosoguanidine (MNNG) and dimethylhydrazine (DMH). Antigenotoxic properties could be due to bacterial metabolites or to cell components. Pellets of stationary *L acidophilus* cultures were supplemented with fresh medium to generate such metabolites and were effective antigenotoxic samples. Also an acetone extract isolated from the pellet culture was effectively antigenotoxic *in vitro*. Putative metabolites of LAB and of other beneficial bacteria (acetate, 2 isomers of lactate, butyrate, palmitic acid, *iso*-palmitic acid, cystein, glutathione) were investigated for antigenotoxic effects in rat colon cells, as were whole freeze dried cells, cell wall skeleton, cytoplasm and peptidoglycans. Some metabolites (acetate, butyrate, cystein, glutathione) did reduce the extent of genotoxicity induced by MNNG, as did peptidoglycans and whole freeze dried cells. Thus, one conceivable mechanism of protective activity by beneficial microflora could be the production of metabolites which inactivate carcinogens in the gut lumen prior to reaching the colon cells.

1. B. L. Pool-Zobel, C. Neudecker, I. Domizlaff, S. Ji, U. Schillinger, et al, Nutr Canc **26**, 365 (1996)
2. B. L. Pool-Zobel, S. L. Abrahamse, G. Rechkemmer, Protective effects of short-chain fatty acids on early events of carcinogenesis: Antigenotoxic effects of butyrate in rat and human colon cells (1996). Proceedings of COST 92, Helsinki Finland *in press*

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APPLICATION OF THE COMET ASSAY TO STUDY OXIDATIVE DNA-DAMAGE IN HUMAN LYMPHOCYTES AND HUMAN COLON CELLS

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Damage to the DNA is involved in at least two major human problems, aging and cancer. Reactive oxygen species (ROS) may produce such types of DNA damage. For clarifying mechanisms and processes of carcinogenesis, it is important to quantify the levels of oxidative DNA damage in tumour target tissues. Using an elegant modification of the Comet assay with endonuclease III treatment (1,2), we have compared DNA single strand breaks (DNA SSB)and oxidised DNA bases in lymphocytes, intestinal tumor cells and in primary colon cells from humans (3). The levels of DNA SSB were higher in primary colon cells > intestinal tumor cells > lymphocytes. Levels of oxidised DNA bases were higher than DNA SSB in each of the cell types and the differences between the types followed the same sequence. To our knowledge, this is the first report on DNA SSB and oxidised DNA bases in human colon cells. Damage is governed by endogenous factors such as DNA repair, antioxidant systems, and by levels of exogenous oxidants. In the future, it will be of foremost interest to study the modulation of DNA damage in colon cells following dietary intervention. This approach promises the development of a new and highly relevant biomarker technique to directly study the impact of specific food ingredients on colon cancer induction and prevention.

1. A. R. Collins, S. J. Duthie, V. L. Dobson, *Carcinogenesis* **14**, 1733 (1995).

2. A. R. Collins, M. Ai-guo, S. J. Duthie, *Mutation Research* **336**, 69 (1995).

3. B. L. Pool-Zobel, A. Bub, G. Rechkemmer, *Proceedings* AOCS Annual Meeting "in vitro & In vivo techniques to detect oxidants and antioxidants", ed. O. Aruoma (1996). *in press*

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PHYSIOLOGICAL FUNCTIONS OF ANTHOCYANIDINES

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Anthocyanines are common coloured plant flavonoids, occurring as glycosides of the respective anthocyanidin-chromophores (1). They have been implicated in contributing to the "French paradox" due to the antioxidative capacity of red wine (2). Like other flavonoids, anthocyanidines are also expected to have antioxidative, antimutagenic and anticarcinogenic properties *in vivo,* although only few data on physiological functions of food derived derivatives are available (3). For the concept of colon cancer prevention, we have therefore begun a series of investigations to study the effects of these compounds on early processes of tumorigenesis in human colon cells. We used *Aronia melanocarpa* Elliot anthocyanidine (AA) -extracts isolated by column chromatography (yield: 1000 ml extract with 16 mg AA/ml from 2 kg fruit). The extracts were used to treat cells of primary human colon biopsies (to study events related to initiation of cancer) and of the human tumor cell line HT29 clone 19A (to study events of signal transduction during progression of tumors). In primary colon cells, high doses of the AA (50-400 μg/ml) were genotoxic, whereas low doses (25 μg/ml) prevented DNA damage induced by H_2O_2, as detected with the Comet assay (4). At still lower doses (3.125-6.25 μg/ml) a significant reduction of cell metabolism was observed in HT29 cells, as determined with a CYTOSENSOR MICROPHYSIOMETER (5). This reduction was due to impairment of neurotensin- and insulin- stimulated acidification, implying interference of both G protein- and receptor tyrosin kinase -linked signalling pathways.

1. J. Kühnau, *World Review on Nutrition and Diet* **24**, 117 (1976).
2. S. Renaud, M. DeLorgeril, *The Lancet* **339**, 1523 (1992).
3. G. Rechkemmer, B. L. Pool-Zobel DGQ-Proceedings (1996)
4. B. L. Pool-Zobel, U. Leucht, *Mutation Res* (1996) *in press.*
5. H. M. McConnell, J. C. Owicki, J. W. Parce, D. L. Miller, G. T. Baxter, et al, *Science* **257**, 1906 (1992)

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STUDIES OF ENZYME ACTIVITIES DURING PROCESSING OF PAPRIKA (CAPSICUM ANNUUM L.)

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Paprika is an important spice and considerable amounts are used in various food preparations. The main quality criteria is the colour of the finel product. Hungarian paprika which is imported in to the Federal Republik of Germany is a rather colour intensive product in the case of production starting from the post harvest treatment until the final preparation of the powder is correctly done. Main items in the production of high quality paprika powder is the correct post harvest ripening, if possible in boxes with good air circulation, artificial drying at moderate air temperatures, grinding in appropriate mills and packaging in light tight material. Factors influencing the deterioration besides the process conditions are the enzyme content and the water content after the drying process.

An analysis of enzyme activities shows maximum enzyme activities of peroxidase (POD) and polyphenoloxidase (PPO) in calyx, stem and seeds.

Drying temperature and grinding (hammer mill) influence enzyme activities and ASTA (colour) values strongly.

A comparison of end products obtained from two different processing lines (institute A: gentle processing; plant B: industrial process) reveals large differences in product quality.

HUMAN INTERVENTION STUDIES IN THE DEVELOPMENT OF FUNCTIONAL FOODS: THE CASE OF NON DIGESTIBLE OLIGOSACCHARIDES.

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Functional food is defined as a "Food which contains, in adequate concentration, one or a combination of component(s) which affects functions in the body so as to have positive cellular or physiological effects which may, in due course, justify functional or health claims". The research and development of functional foods is the objective of "Functional Food Science", a part of the science of Nutrition. Various concepts are presently being developed in "Functional Food Science" with regard to ways of preparing such foods but most importantly with regard to strategy for research and development. Such a strategy involves:

1. identification and, possibly, the understanding of the mechanism of an interaction between a food and function(s) in the body leading to functional effects;
2. development of sound hypothesis as well as relevant methodologies to investigate, in humans and in relevant protocols, such functional effects as well as their consequences;
3. identification of effects supporting functional or health claims which need to be approved based on relevant scientific data.

The non digestible oligosaccharides (NDOs) are new food ingredients which belong to the class of non a-glucans. They are fermented by the colonic bacteria and, at least for some of the NDOs, such a fermentation is highly selective and leads to a profound modification of the composition of this microbiota. One example of such an effect is the stimulation of bifidobacteria by the chicory fructooligosaccharides. Based on good experimental evidences, the bifidogenic effect has been demonstrated in human intervention studies and bifidogenesis is a recognised functional claim for this NDO.

Chicory fructooligosaccharides have also been shown, in experimental systems, to interact with hepatic lipogenesis and a sound mechanistic hypothesis has been proposed to explain such an effect. Thus, relevant human intervention studies can be designed to test this hypothesis. But aimed at demonstrating a functional effect of a food as part of an otherwise balanced diet, such

a design should meet specific rules. Indeed, a functional food is not a drug, it is not aimed at curing a specific disease but rather at helping the "general" population to improve health and, eventually, to prevent pathological processes.

Other preliminary experiments have shown that the chicory NDO could intertact with other functions in the body, like absorption of ions or even prevention of carcinogenesis and retardation of tumor growth. In the absence of sound scientific hypothesis, such observation cannot yet be used to justify human intervention studies.

Human intervention study is a key element of functional food development because it is essential to the demonstration of an effect to justify a "claim". Such a study requires an appropriate design but such a design can be made only to test a sound hypothesis supported by solid scientific data.

MODULATION OF INTESTINAL BACTERIA BY COMPLEX CARBOHYDRATES AND COLON CANCER PREVENTION

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The biological significance of changes in the gut microflora induced by dietary carbohydrates, particularly changes relating to colon carcinogenesis, is the subject of research currently being conducted at BIBRA in collaboration with the Institute of Nutritional Physiology , Karlsruhe, and the Universities of Florence and Camerino. The studies encompass work on microflora composition, microbial formation of carcinogens, genotoxins and tumour-promoters and the modulation of early changes in the colonic mucosa considered to be indicative of cancer risk.

a) Modulation of gut microflora composition by complex carbohydrates

Carbohydrates that are poorly digested in the upper gastro-intestinal tract , for example non-starch polysaccharides (NSP), resistant starch (RS) and non-digestible oligosaccharides (NDO) can reach the colon where they may be used as a carbon-energy source by the resident microflora. We have demonstrated that NDO and RS consumption is associated with an increase in lactobacilli and bifidobacteria in the colon. Such studies, while indicating that a major change in the microbial eco-system has occurred give no indication as to its biological significance. Consequently further studies were performed with more direct relevance to colon damage and cancer.

b) Modulation of activity of enzymes associated with carcinogen formation and activation

The microflora of the intestinal tract has a broad range of metabolic activities which can result in the synthesis or activation of carcinogens, genotoxins, tumour promoters and anti-carcinogenic agents. Reactions that we have studied in our research programme include: *Synthesis of 7-OHIQ*

Incubation of the cooked food component IQ with a suspension of human faeces yields the 7-keto derivative, 7-OHIQ, which exhibits genotoxic activity towards colon mucosa cells *in vitro*. Feeding of NDO and RS to rats was associated with a significant decrease in the rate of conversion of IQ to 7-OHIQ in caecal contents *in vitro*.

The bacterial enzyme β-glucuronidase (an enzyme associated with many common gut organisms), can hydrolyse biliary conjugates of carcinogens releasing the parent compound, which can then interact with the gut mucosa.

Supplementation of rat diets with NDO was usually associated with a decrease in caecal activity of β-glucuronidase. Similarly, the presence of starch, digestible or amylase-resistant, decreased the β-glucuronidase activity.

c.) *Effectscts of carbohydrates on biomarkers of cancer risk*: Conventional cancer bioassays are too expensive and time-consuming to allow screening of food components for modifying effects on carcinogenicity and do not facilitate studies of mechanisms of action. To circumvent these problems, we are evaluating the protective effects of NDO and RS by utilising a variety of rapid techniques considered to be predictive of risk of neoplasia in the colon.

d.) DNA damage in the colon (Comet assay): Rats fed the RS Crystalean (a retrograded amylomaize starch) exhibited decreased levels of carcinogen-induced DNA damage in the colonic mucosa by comparison to rats given diets containing sucrose, digestible starch or soy fibre. Similar protective effects against colonic DNA damage were apparent when rats were fed the synthetic disaccharide lactulose. The latter is not digested in the upper gut and so reaches the colon where it has been shown to modify the resident microflora.

b) Induction of pre-neoplastic lesions (aberrant crypt foci) in the colon.
Aberrant crypt foci (ACF) are putative, pre-neoplastic lesions in the colon. They are formed when rats are treated with carcinogens known to target the colon eg 1,2-dimethylhydrazine and azoxymethane (AOM). We have investigated the influence of feeding dietary inulin (a source of a mixture of NDOs of various chain lengths) on ACF induction by AOM in rats fed either a low fat or high fat diet. Significant effects on ACF numbers of the inulin treatment was seen, with the protective effects being more potent in the animals given the high fat diet.

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FLAVONOIDS IN UK TOTAL DIET SAMPLES

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Four flavonoid compounds (kaempferol, quercetin, apigenin and luteolin) were determined in 120 UK Total Diet Samples. Total Diets are a reconstruction of the average UK diet, split up into 20 groups of similar foods. Analysis was carried out on those groups likely to contain flavonoids: Green vegetables; Canned vegetables; Other vegetables; Fresh fruit; Fruit products; and Beverages. Wine was also analysed because it may contribute to intake.

The flavonoids are one of several groups of chemicals which occur naturally only in fruit and vegetables and which, although not essential for the maintenance of health, are considered to have beneficial properties. The compounds examined form only a small proportion of the total flavonoids occurring in foods. Other types of flavonoids (such as catechins and procyanidins) occur at much higher concentrations and may be of greater significance for human health.

Flavonoids occur in food as a complex mixture of glycosides. Extracts were subjected to acid hydrolysis and the sum of the individual parent aglycones determined. This is the same approach adopted in the only other available study of intake, based on analysis of individual fruits and vegetables in the diet of a population from The Netherlands. It was found that:

The calculated intake of apigenin, luteolin, kaempferol and quercetin by UK consumers is 30 mg d-1, broadly in line with that estimated for a Dutch population, although higher. Quercetin accounted for 64% of the total. The Beverage Total Diet group provided the bulk (82%) of the intake.
Wine makes a negligible contribution to the intake of the average wine consumer.

EFFECTS OF DIFFERENT RESISTANT STARCHES IN THE NUTRITION OF RATS.

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Apparently 50% of all colorectal cancer cases in Germany are nutrition dependent. Their incidence increases. One cause of this may be the low content of dietary fiber in nutrition of industrialized countries. Our investigations have been focused on structure and function of resistant starches (RS). Rats were fed with different types of RS over a period of 77 days. From the results can be concluded that the major effects of RS are an increase of fecal weight and frequency of defection, a decrease of bile acid dehydroxylation and an enhancement of microbial growth. Last property depends on the structure of RS. The highest rate of microbial metabolism was observed with maltodextrine products. They produce the highest rate of short chain fatty acids, especially butyrate, which is substrate of the energy metabolism and an important metabolite for special cellular functions of colonocytes.

PHYTIC ACID AND INOSITOL PHOSPHATES DURING GASTRO-INTESTINAL DIGESTION IN PIGS

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Phytate, the hexaphosphate ester of myo-inositol, is widely spread in the plant king-dom. Its highest levels occur in plant seeds as cereals and legumes (0.5-5% of DM). During gastro-intestinal digestion phytic acid (IP_6) is degraded in part and inositol phosphates which do not inhibit the intestinal absorption of trace elements and minerals as Zn and Fe are formed. Moreover, biologically active inositol phosphates as Ins(1,4,5)P_3 may also arise. As the mechanisms of enzymatic hydrolysis of IP_6 during digestion have not yet been elucidated and the degradation products have not yet been determined completely, pigs (n=4) were fed with a diet rich in phytic acid and samples from stomach, jejunum and ileum were analyzed for IP_6 and the other inositol phosphates IP_5, IP_4, IP_3, IP_2, IP_1 using an ion exchange HPLC method.

In the content of stomach IP_6 together with nine other inositol phosphates were determined. Due to a preliminary evaluation of the results the predominant inositol phosphates are Ins(1,2,3,4,5)P_5, Ins(1,3,4,5)P_4, Ins(1,2,3,5)P_4 and IP_3. Two other IP_5 isomers [Ins(1,2,4,5,6,)P_5 ; Ins(1,2,3,4,6)P_5] and one more IP_3 isomer [Ins (1,5,6)P_3] as well as traces of IP_2 and of IP_1 are present in the stomach. In the jejunum the same inositol phosphates as in the stomach occur, however, in lower concentrations. The pattern of the different inositol phosphates stays unchanged. The same has been observed for the inositol phosphates of the intestinal content in the ileum. The results indicate that IP_6 mainly is hydrolysed in the stomach by the plant enzymes of feed. Due to the unchanged pattern of inositol phosphates in the intestinal contents of jejunum and ileum it is assumed that no further enzymatic degradation in the small intestine takes place. About 2/3 of the inositol phosphates formed are IP_4, IP_3, IP_2 and IP_1 , which do not interfere with the intestinal absorption of Fe and Zn. Whether biologically active inositol phosphates arise is not yet clear.

IMPACT OF PROCESSING ON THE RETENTION OF PHYTOCHEMICALS

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Phytochemicals are currently of special interest due to their multiple physiological functions and their potential health benefits. The impact of processing on phenolic-substances, carotinoids and other substances are demonstrated at the example of processes which are at the moment under investigation at the Institute of Process Engineering.

Special emphasis is given to process conditions which allow the retention of phytochemicals during processing and subsequent storage.

The individual processes described are the production of sultana grapes, the drying and grinding of paprika, the dehydration of onions and the preparation of fresh cut green vegetable products such as salads with an improved shelf life.

QUANTITATION OF CAROTENOIDS IN FOOD AND HUMAN SERUM

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Carotenoids are natural colorants present in various fruits and vegetables, such as carrots, tomatoes, spinach, oranges, or peaches. Epidemiological studies have shown that the increased consumption of foods rich in carotenoids is correlated with a diminished risk for several cancers. Thus, the carotenoid pattern in fruits and human serum as well as the bioavailability of these compounds from natural sources is of considerable interest. Cryptoxanthin is the predominant carotenoid in citrus fruits, mainly present in esterified form. More than 30 different carotenol esters have been indentified in orange and tangerine juice applying MALDI-TOF mass spectrometry (1). Recently, we demonstrated that these esters considerably contribute to the supply with cryptoxanthin in the human (2). However, even after ingestion of a source high in cryptoxanthin esters, only the level of the free carotenoid increased in human serum and chylomicrons. Apart from cryptoxanthin, ß-carotene, α-carotene, lycopene, and lutein are the major carotenoids in human serum and tissues. Several tissues such as liver, adrenal, or testes are rich in carotenoids while lower amounts are detected in kidney, ovary, or brain (3).

The antioxidant properties of carotenoids and their ability to induce gap junctional communication have been discussed as biochemical mechanisms underlying their cancer preventive effects. Carotenoids are efficient quenchers of singlet oxygen; their activity depends mainly on the number of conjugated double bonds present in the molecule. A variety of natural occuring carotenoids induces intercellular communication via gap junctions, a process which is likely to be relevant for the growth control of transformed cells (4).

References:

1. Wingerath T., Stahl W., Kirsch D., Kaufmann R, Sies H. (1996) J Agric Food Chem 44, 2006

2. Wingerath T., Stahl W., Sies H. (1995) Arch Biochem Biophys 324, 385

3. Stahl W., Schwarz W., Sundquist AR, Sies H. (1992) Arch Biochem Biophys 294, 173

4. Stahl W., Hanusch M., Nicolai S., Briviba K., Broszeit G., Peters M., Martin HD, Sies H. (1996) Carcinogenesis, submitted

PROANTHOCYANIDINS OCCURRENCE AND ANALYSIS IN FRUIT AND VEGETABLES

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Proanthocyanidins, also named condensed tannins, belong to the flavonoids which are a widespread group of secondary compounds in plants. The proanthocyanidins are well known because of their astringent properties due to the precipitation of proteins. In plants, the proanthocyanidins play an important role in the resistance to various diseases and environmental stress. Therefore, they are localized in specialized cells of bordering tissues such as epidermal and bark cell layers or glands both in the central vacuole and bound to the cell wall. Besides, their precipitating activity the, condensed tannins possess antioxidant and radical scavenging properties. The efficacy of these characteristics depends on the number of OH-groups, on the size of the individual molecule and its sterical configuration. Because of that it is useful to estimate the individual content of the different proanthocyanidins occurring in fruit, fruit juices and vegetables. Instead of the old colorimetric methods measuring the total proanthocyanidins, an analytical procedure is presented using reversed-phase high-performance liquid-chromatography combined with post column derivatization by p-dimethylaminocinnamaldehyde.

QUANTIFICATION OF GLUTATHIONE -TRANSFERASE SUBUNITS IN RAT COLON CELLS BY HPLC AND COMPARISON TO CORRESPONDING LIVER DATA

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The Glutathione S-Transferase isoenzymes (GSTs) are multifunctional phase II-enzymes, which may protect against cancer by inactivating carcinogens. Subunits may be determined by Western blotting or HPLC. Thus, using the former method, we have shown rat colon cells to contain predominantly subunit π followed by μ and α. We have now performed a comparative analysis of the different subunits in rat colon and liver cells of the same rat using HPLC. The cell number was taken as a basis to compare GST subunit levels in the two tissues. We observed that the total amount of GSTs in liver was higher than in the colon; different patterns of subunits occur in rat liver (main subunits: 1,2,3,4) and in colon cells (subunits identified in most rats: 7,4,3,2). The differences were within and between groups of rats fed a semi purified diet for different lengths of time. A lower interindividual variation of GST content was observed when evaluating the data based on the cell number than when taking the protein content as basis for calculation. The analysis according to the cell number is expected to yield results which more sensitively reveal modulation by external factors.

References

[1] Nijhoff, W.A., et al.: Int. J. of Onc. Vol. 3, pp. 1131-1139, 1993.

[2] Bogaards, J.J.P., et al.: Fd. Chem. Toxic. Vol. 28, No. 2, pp. 81-88, 1990.

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IMMUNOMODULATORY ACTIVITY OF PHYTOCHEMICALS

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The significance of nutrition as a key factor in host defense has become evident during the last decades. However, the specificity of this interaction at the level of the immune system is still unclear. In industrialized countries nutrition affects the immune system mainly by supplying a surplus of energy and fat and by an inadequate intake of vitamins and minerals. The increasing knowledge on the contribution of the immune system to the pathogenesis of common diseases like cancer, cardiovascular diseases and AIDS as well as allergies has stimulated studies to modulate the immune system by nutritional intervention.

In contrast to the many studies on the impact of nutrients on the immune system, the impact of dietary non-nutritive factors like phytochemicals on the human immunocompetence have not been studied well. The carotenoids are the only group of phytochemicals, which have been intensively investigated in animal as well as in human studies. The main emphasis has been on the effects of ß-carotene, and few studies have looked at carotenoids without provitamin A activity, such as canthaxanthin and astaxanthin. ß-Carotene affects phenotype expression of lymphocytes in humans and enhances effector functions, like natural killer cell activity, lymphocyte proliferation and delayed type hypersensitivity. The flavonoids are another major group of phytochemicals, of which individual compounds show immunomodulatory activity. Various animal studies indicate thet they inhibit inflammatory, proliferative and allergic activities. Most of the flavonoids studied in animals so far exerted immunosuppressive effects. Based on this knowledge flavonoids have been used in animal studies to counteract an overreacting immune system. However, in most of these studies flavonoids have been applied parenterally and studies with humans are still missing. Flavonoids could affect the immune system by inhibiting key enzymes important for the synthesis of endogenous mediators of the immune response as well as by interfering with signal transduction factors like NF_kB.

Further groups of phytochemicals, for which in vitro and animal data indicate an immunomodulatory activity are the saponins, the sulfides and phytic acid. However, the current data are rather poor and additional studies are necessary to identify the clinical significance of these compounds for the immunocompetence of healthy subjects. In conclusion, certain

MODULATION OF PUFA BIOSYNTHESIS BY DIETARY PETROSELINIC ACID
(ω12-Octadecenoic acid) from coriander oil

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Petroselinic acid is an unusual monoenoic C18 fatty acid with a cis C=C double bond in Δ6 position; it is a positional isomer of the more common oleic acid (ω9-octadecenoic acid). The seed oils of parsley, coriander and other plants of the family Umbelliferae which are commonly used as kitchen spices contain high proportions of petroselinic acid. We have fed coriander oil over a period of ten weeks to rats and studied the physiological and metabolic effects of this petroselinic acid-rich seed oil. Our results are as follows: 1. Feeding of high doses of coriander oil leads to degenerative alterations (fatty cysts) in rat liver. 2. High amounts of petroselinic acid are incorporated into triacylglycerols and phospholipids of various organs and tissues of rats during the feeding period. 3. In the livers of animals petroselinic acid is elongated to Δ8-eicosenoic acid as well as catabolized by β-oxidation to Δ4-hexadecenoic acid. The formation of PUFA from petroselinic acid, however, was not observed. 4. Δ6-desaturation and elongation reactions of linoleic acid (ω6) are inhibited in the livers of rats in the presence of petroselinic acid leading to reduced levels of arachidonic acid (ω6) particularly in phospholipids of liver and heart.

It is envisaged that petroselinic acid having a Δ6 double bond mimics a product of Δ6-desaturase and thus induces 'pseude-product' mediated inhibition of desaturase which finally lowers arachidonic acid concentration in membrane phospholipids. It is conceivable that such reduction can be utilized therapeutically, e.g. to modulate the formation of specific eicosanoids in the treatment of certain diseases.

ANION EXCHANGE PROCESS FOR SELECTIVE NITRATE REMOVAL FROM LIQUID VEGETABLE PRODUCTS

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Nitrate selective anion exchange resins primarily developed for the treatment of water supplies were successfully applied to nitrate removal from more complex systems like liquid food products or processing media. This new application is a promising alternative to microbiological denitrification.

Experiments to determine selectivity of these anion exchange materials were conducted in 7 major anion species present in vegetables. The exchangers tested prefer nitrate to all other anions. For all exchangers tested the following selectivity sequence was obtained:

Nitrate > Oxalate > Malate > Nitrite > Sulfate > Chloride > Phosphate

Hence, both nitrate and oxalate could possibly be removed.
Exchange equilibria were described by a theoretical approach that allows the prediction of multicomponent equilibria by parameters of binary equilibria. There is an excellent agreement between predicted and experimental data.

Breakthrough experiments in a complex vegetable extract (spinach blanching broth) confirmed the selectivity sequence derived from binary equilibria studies. It may be concluded that this vegetable extract does not contain substances of higher affinity to the exchanger than nitrate.
Fouling caused by adsorption of organic substances on the ion exchanger materials did not reduce exchanger performance over repeated cycles. Adsorption has been found to decrease with increasing numbers of exchange/regeneration cycles.

BENEFITS AND RISKS OF DIETARY GLUCOSINOLATES AND FLAVONOIDS

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Fruits and vegetables in the diet reduce the risk of chronic diseases by up to 50%. There are several mechanisms currently under investigation which may contribute to this effect. The antioxidant hypothesis is currently popular, and may be a major mechanism for protection by the diet against heart disease, cancer and cataract.

The antioxidant action of food components can arise directly, by virtue of the direct free radical scavenging properties of compounds such as vitamins E and C, carotenoids and phenolics. The effect can also be indirect, by stimulation of endogenous antioxidant/detoxifying enzymes. Examples of these enzymes are glutathione S-transferases, glutathione peroxidases, quinone reductase, UDP-glucuronosyl transferases and superoxide dismutase.

Flavonoids are present in high amounts in many diets and a number of physiological effects have been ascribed to this class of compounds. However, much of this work has been on flavonoid aglycones, whereas the form of flavonoids inthe diet isalmost exclusively as glycosides. The properties of these glycosides are compared to the aglycone, and it is shown that there is a large difference in properties associated with antioxidant action.

Compounds in the diet which stimulate defences are certain sulphur compounds in Brassica and Allium vegetables, as well as some antioxidants and some phenolics. In Brassica vegetables (broccoli, Brussels sprouts, cabbage, cauliflower, mustards), the major class of bioactive components are glucosinolates. In Alliums (onions and garlic), the major class are the cysteine sulphoxides. The effect of these compounds is to stimulate enzymic defences via the antioxidant responsive element which is present in the 5 - flanking region of many of the above enzymes.

APPLICATION OF PRIMARY RAT COLON CELLS FROM BASAL AND SURFACE CRYPT-SECTIONS FOR PROLIFERATION AND GENOTOXICITY STUDIES

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Increased colon cell proliferation, especially in the basal crypt-sections, and genotoxicity, (leading to mutations in tumour oncogenes and suppressor genes,) are two parameters which contribute to the early steps of colon carcinogenesis. In order to study the impact of nutritional factors on these parameters, our efforts have been focused on establishing an isolation technique to obtain rat colon cells in different stages of differentiation. Rat colon cells were isolated stepwise from the villus tip to the basal crypt using a modification of the method of Schulman et. al., (Am.J.Physiol. 266 (Cell Physiol. 35): C729 - C740, 1994). These fractionated cells were then employed to study endogenous levels of DNA-damage, oxidized DNA-bases and proliferation rates. Additionally, the potential protective effects of short chain fatty acids (SCFA) on *in vitro* proliferation were examined.

We found that cell yield increased from fraction 1 to 6 and total cell yield of all fractions was 61 ± 25 millions. Viability, determined by trypan blue exclusion, was between 70 and 86%. The basic rate of cells with intact DNA from fraction 2,4 and 6, as measured by single cell microgelelectrophoresis (comet assay) was 35%, 47% and 53%, respectively. The degree of endogenous oxidative DNA-damage, visualized by treatment with endonuclease III, was increased by about 8% in fraction 2, 10% in fraction 4 and 26% in fraction 6. The proliferation rate evaluated by bromodeoxyuridine-incorporation and detection of proliferation nuclear antigen (both detected immunohistochemically) was not different in fraction 3 and 6 following 1 hour 45 minutes or 2 hours 45 minutes incubation. Furthermore, treatments by butyrate or acetate (6.25 mM) did not alter this parameter.

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