Nutrition, Residues and Health

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Going back at least 30 000 years human beings have consumed meat, primarely from wild animals until the practice of domestication of animals has been gradually accustomed. In addition, different methods of meat preservation have been developed for centuries, including drying, smoking, salting, sausage making and even cooling of fresh meat in northern climates. Meat science and technology as specialized areas in the field of nutrition are young, still developing disciplines. They require an interdisciplinary approach to cover chemical, physiological, biochemical, genetical, microbiological, nutritional and technological aspects.

Reviewing decade-to-decade advances in the nutrition field in the past 50 years, dramatic changes become evident. The *discovery era*, during which fundamental knowledge on essential nutritional factors such as vitamins and trace elements emerged, is gradually followed by a *biochemical function era*. The latter is characterized by fundamental research activities to assess the impact of nutrients on modulation of enzyme activities, turnover or degradation of extracellular, intracellular, cell surface and nuclear binding proteins and - more recently - their effect on genetic expression and regulation of metabolic sequences in somatic cells (ERDMAN, 1989).

Whilst scientists still try to amand the understanding of all facets involved in health and nutrition, public interest focusses on adverse effects of certain foods. Emerging concern about the consumption of (red) meat led to a critical perspective on production methods (animal genetics, feeding and husbandry), the nutritional value and potential risk factors associated with meat consumption. These include overnutrition, processing methods, food additives and residues from environmental pollutants or agro-chemicals. As a consequence, an imbalanced discussion on diet-health relationships results in numerous recommendations for a *healthy* diet. It has to be stressed that only a strict application of well evaluated scientific methods can resolve the specific interrelationship between dietary pattern and incidence or prevention of disease (HARPER, 1988).

MEAT IN THE DIET

Proteins

Meat, i.e. the flesh of domestic animals including poultry and game has always been regarded as an important source of protein in human nutrition. The requirements for total protein in various stages of the life cycle have been reviewed by FAO, WHO, UNU (1985). These requirements for dietary protein consist of two components:

(1) the requirement of nutritionally indispensible (essential) amino acids and conditionally - under specific physiological or pathological conditions - indispensable amino acids, and
(2) the need to meet the requirements for non-specific nitrogen to supply the nitrogen necessary for dispensable amino acids in protein synthesis and other nitrogen containing compounds under physiological conditions.

The discussed requirements focus on maintenance. In the last four years human amino acid (protein) requirements have been a subject of intensive debate (YOUNG, 1987; BEATON and CHERY, 1988; MILLWARD et al., 1989; MILLWARD and PRICE, 1990). As recently summarized by MILLWARD et al. (1989), the existing N balance findings include several disparities between individual studies namely:

* the shape of the N intake - N balance response curves,
* the magnitude of the intake allowing apparent equilibrium,
* the difficulty of demonstrating equivocal differences in N balance responses between dietary proteins which differ in their indispensable amino acid content and hence protein score,
* the existence of statistically identificable cyclic patterns of N excretion and balance in some long-term balance studies but

not in others,

* the occurrance of enzyme changes thought to be pathophysiological in some long term balance studies but not in others,
* the existence of inverse correlations of urinary to faecal N excretion in some studies but not in others.

MUNRO (1985) has described, that concerning high quality protein - such as meat - the organism utilizes it with perfect efficiency to provide for obligatory needs. As the equilibrium is approached, excretion increases to match any further intake. However, the described new understanding of protein requirements may imply revised estimates of the nutritional value of certain protein sources (REEDS, 1990). As amino acid composition of various meats and meat products may vary and can be influenced by processing such as canning and careful dehydration, the increasing knowledge about protein requirements should be used to design modern concepts in dietetics for healthy and diseased people (YOUNG, 1989).

Fat and Cholesterol

In contrast to meat protein which is generally accepted to be of nutritional value, albeit the above mentioned discussion upon protein requirements, animal fats available in meat and particularly in meat products are a subject of controversy. The fact that the intake of excessive fat, over one's caloric needs, is an important risk factor in cardiovascular disease, hypertension, stroke, diabetes, obesity and/or specific types of cancer is well accepted. The charges that *saturated fats* are main sources of excessive and harmful calories and are hypercholesterolemic and thus atherogenic have caused an actual phobia against consumption of meat fat. Conventional wisdom has translated *saturated fatty acids* to mean *saturated fat* and that meat fat is all saturated.

Meat fat in beef and pork contains predominantly palmitic acid C16:0 (25-30%), stearic acid C18:0 (24-29% in beef; 12-16% in pork) and minor amounts of lauric acid C12:0 and myristic acid C14:0. In addition, high concentrations of oleic acid C18:1 (41-51%) and varying amounts of linoleic acid C18:2 (2-3% in beef; 6-12% in pork) and linolenic acid C18:3 (0.5-1%) and arachidonic acid C20:4 (0.1% in beef; 0.2-2% in pork) can be measured. Traces of 5- and 6-double bond fatty acids have been detected in poultry and game ruminants. Genetic selection of pigs with a high muscle mass tends towards a selection of soft fat containing a high amount of unsaturated fatty acids. Meat technology favours saturated fatty acids, however, nutritionists stress the desirable biological effects of polyunsaturated fatty acids.

Since every fatty acid has its own metabolic pathway, meat fatty acids elicit physiological and biochemical responses similar to those of vegetable oils, although with some quantitative differences. The impact of dietary fatty acids on coronary heart disease (CHD) has been an item of intense and controverse discussion since years. One of the most reasonable explanations for the numerous reports about diet composition and disease is the fact that the complex network of lipid absorption and transport, the regulation of endogenous and exogenous cholesterol and the impact of capacitatively limited cholesterol catabolism and excretion is not completely understood. Results on the correlation between diet and CHD are based either on animal experiments or on retrospective epidemiological studies. Both are of limeted value for an overall evaluation, as animal experiments cannot reflect the speciesspecific differences in lipid metabolism and retrospective epidemiological studies often lack essential information about the pattern of fatty acids consumed with the diet, and other (social) factors which might contribute to pathologically elevated cholesterol levels.

Based on overall experience current recommendations limit intake of *saturated fatty acids* to not more than 10% of one's caloric intake. However, it has to be noticed that as for example stearic acid, one of the most common saturated fatty acids in many animal fats, can be converted to oleic acid, the most abundant monosaturated fatty acid (BONAMONE and GRUNDIG, 1988). Oleic acid increases the desirable high density lipoprotein (HDL) fraction (WALLACE and ANDERSON, 1987). The proportion of saturated fatty acids consumed is considered to be responsible for elevated blood cholesterol levels. However, the increase is not a straight line relationship but is curvilinear or in steps. Between 6 and 13% of saturated fatty acid calories, the serum cholesterol level remains reasonable constant at about 200 mg/dl. At a point between 13 and 18% of dietary saturated fatty acids, the homeostatic mechanism appears to break down and serum cholesterol increases.

The ability of (poly)*unsaturated fatty acids* to decrease plasma cholesterol levels has already been recognized 30 years ago. However, previously recommended high linoleic acid-rich diets are now disapproved. Even though linoleic acid may decrease the risk of CHD by decreasing atherosclerosis, it may at the same time enhance CHD by increasing thrombosis, and there is increasing evi-

dence that CHD is mainly a thrombotic event that takes place against an atherosclerotic-hypercholesterolemic background induced by thromboxane A_2 synthesized from linoleic acid in the platelets (REISER and SHORLAND, 1990).

Meat tissue lipids contain also eicosanoid *omega*-3 fatty acids, which seem to reduce the synthesis of thromboxane A_2 and increase the level of thromboxane A_3 and prostacyclin. These changes have been reported to decrease platelet aggregability, resulting in decreased clotting time and a decreased risk for atherosclerotic plaques. *Omega*-3 type polyunsaturated fatty acids occur in lipoprotein phospholipides in all membranes. However, the concentration in meats varies according to age, sex, breed and nutrition of distinct animals. *Omega*-3 type fatty acids (i.e. eicosapentaenoic acid C20:5 (EPA) and docasahexaenoic acid C20:6 (DHA) are now discussed to have a therapeutical potential in prevention and treatment of a number of diseases, including CHD, stroke, hypertension, auto-immune disorders and cancer (von SCHACKY, 1987).

Hypercholesterolemia is one of the few independent risk factors definitively linked to increased morbidity and mortality due to myocardial infarction. Cholesterol enters the body pool from two sources, either by absorption from the diet or by endogenous synthesis. Endogenous cholesterol synthesis ranges from 11-13 mg per kg body weight, resulting in a total daily amount of 770-910 mg cholesterol. Dietary cholesterol intake resembles to approximately 450 mg/day in Western diets of which about 60% (270 mg) is absorbed. Thus, the total body cholesterol turnover equals 1110 mg/day: 24% from diet and 76% from synthesis (McNAMARA, 1987).

Meat - in a Western diet and in all forms and types - provides about 34% of the cholesterol in our diet (BLOCK et al., 1985). At this level it may be that the cholesterol in meat products adds between 3-4 mg/dl to plasma cholesterol levels, or approximatively 1.5-2% of the total. Thus, the rationale of labeling meat products as *bad foods* based on their direct contribution to elevated plasma cholesterol levels seems unnecessary and unreasonable (McNAMARA, 1990). Plasma cholesterol levels are mediated by a variety of nutritional (total fat, fibre intake) and non-nutritional factors (genetic disposition, hypertension, coffee consumption, smoking). Physiological regulation can resolve perfectly nutritional cholesterol intake in healthy persons. In diseased people a careful analysis of all factors contributing to elevated plasma cholesterol concentrations have to be conducted.

CHD is a complex pathophysiological event which involves a three-step process: injury, plaque accumulation, and myocardial infarction. Plaque accumulation is considered to be a consequence of total blood cholesterol levels. However, it has to be pointed out that the first step, the cause of endothelial injury remains still to be elucidated. There is increasing evidence that *lipid peroxidation products*, present in oxidized fats from fatty fish, fat frying and powdered foods may exert atherogenic effects. Fatty acid hydroperoxides have been shown to accelerate all three phases of atherosclerosis: endothelial injury, accumulation of plaque and thrombosis. Higher levels of lipide peroxides are observed in animals and patients with atherosclerosis than in those with no clinical evidence of the disease (KUBOW, 1990).

Thus, albeit the vast amount of studies, both epideminological and experimental, a final scientifically based concept for a diet, considering all aspects contributing to CHD, is still lacking. As a practical approach the National Cholesterol Education Program Expert Panel (NCEP) (1988) has recommended to everyone to limit fat consumption to less than 30% of total calories and to increase carbohydrate consumption to about 50 or 60%. In addition,it is recommended that total dietary fat should not exceed one-third each of saturated, monounsaturated and polyunsaturated fatty acids.

Vitamins and Iron

Meat serves as an important source of many water soluble essential nutrients including the B vitamins. Requirements for B group vitamins may vary according to age, sex, pregnancy, health status and long time usage of drugs (including oral contraceptives) or alcohol abuse.

Thiamine (B_1), riboflavine, niacin, and panthothenic acid are present in meats in concentrations meeting the recommended dietary intake (recommended dietary allowance RDA) per 1000 kcal (for review see WINDHAM, WYSE and HANSEN, 1990). The contribution of Vitamin B_6 and Vitamin B_{12} to average intake *per capita* per day is 46 and 77%, respectively. As Vitamin B_6 and B_{12} losses may be high (up to 75%) in processed and canned meats (and vegetables), analytical values can be taken only from ready-to-eat meals. Meat plays a major role in Vitamin B_{12} supplementation, as cereals - even when fortified - provide only a small percentage of the required amount due to the low bioavailability of the vitamin from these commodities. Strict low calory diets, vegetarism

or a reduced food intake in elderly people might therefore cause deficiencies. Food composition data should provide additional information about vitamin B_6 and B_{12} stability during processing and about the bioavailability of meat supplied vitamins (SAUBERLICH, 1990).

Besides the nutritional relevance of water soluble vitamins present in meat, the role of fat soluble vitamins in meat associated fats have to be mentioned. The value of liver as a source for vitamin A in the diet was conclusively demonstrated by the NHANES II survey data (BLOCK et al., 1985). Liver consumption varies significantly in different populations. According to the USDA Nationwide Food Consumption Survey only 36% of the American population consumes liver, whilst the percentage in Europe is much higher due to a variety of meat specialities prepared with liver tissue. As vitamin A or carotinoid fortified diets have been widely used in animal nutrition a very frequent consumption of liver or chicken meats may result in an excessive vitamin A intake. Vitamin A is known to be teratogenic and very recently a case-report about an infant with malformation due to excessive liver consumption by the mother during pregnancy has implicated an update of vitamin A tolerance in humans and concentrations present in meats.

Meat is not an especially rich source for the other fat soluble vitamins like vitamin E, vitamin D or vitamin K (for review see SMITH, 1990). However, the physiological role of vitamin E as an antioxidant and protective agent in (meat-) fat associated disease and carcinogenesis is a subject of increasing interest.

Amongst the trace elements present in meats **iron** has to be nominated in the first place. No other category of foods provides as high a level of bioavailable iron. Even in high developed countries impaired iron status is common in women of the reproductive stage (WORTHINGTON-ROBERTS et al., 1988). In addition, iron deficiencies in populations with protein-caloric malnutrition have been described, resulting in behavioral abnormalities in iron-deficient infants and preschool children (GRIESEN, 1986). Food iron may be classified as either heme (hemoglobin, myoglobin, cytochromes) or nonheme iron. In human beings heme is well absorbed and released iron enters the body iron pool. All iron in plants and non-cellular animal derived foods such as eggs and dairy products contain only nonheme iron. Rates of absorption vary considerably between heme (15-35%) and nonheme (2-20%) iron. Besides a more effective absorption of heme iron, meats (red meats, fish, poultry) are dietary enhancers of nonheme iron, which could be demonstrated in a comparative approach with semi-synthetic meals (MONSEN, 1988). Albeit various attempts to fortify nutrients with iron or to enhance iron absorption by ascorbic acid, well selected diets of high iron bioavailability will always play an important role to meet more closely the iron needs of the organism.

MEAT AND CANCER

ARMSTRONG and DOLL (1975) compared incidence rates for 14 cancers in 32 countries and reported a positive correlation of meat intake with cancers of the pancreas, breast, colon, prostate and endometrium. PHILIPPS et al.(1983) evaluated epidemiological studies relating cancer risk to meat ingestion. In 11 case-control studies of colon cancer they found six which give a positive association, one to give a negative association and four giving no association. In addition, various comparisons of colon cancer incidence in certain religious groups (consuming none or eat large amounts of meats), provided inconsistent evidence for a direct correlation of meat ingestion and cancer.

Meat provides three nutrients which might be involved in carcinogenicity i.e. protein, fat and cholesterol. Dietary **protein** supplementation is known to influence a variety of physiological functions in the animal body including the immune response. Experimental studies applying different casein concentrations to rodents in order to demonstrate a protein concentration-dependent effect on carcinogen-induced tumours failed to provide significant evidence for an association. However, many studies with rodents have demonstrated inhibitory effects of energy restriction on incidence and growth rate of spontaneous and carcinogen-induced tumours (ROSS and BRAS, 1971). The discussion about the association of meat consumption to cancers of the large bowel (WILLETT, 1989) has to include the relation between dietary fat and cancer, as a differentiation is difficult and investigators have been unable to agree whether the association is specific for colon cancer, rectal cancer or both (DOLL, 1990).

Experimental diets high in **fat** (>100 g/kg) have shown to enhance tumorigenesis in spontaneous, carcinogen-induced, X-irradiation induced and transplantable mammary tumours in both rats and mice (WELSCH, 1987). As the effects of high-fat diets are more pronounced when these are fed after the initiation phase in tumour models, it is concluded that high-fat diets may promote (and not initiate) carcinogenesis. Various studies tried to attribute certain types of fats (concentration of high saturated versus unsaturated

fatty acids) to tumour incidence. IP (1987) found, that tumour incidence is linearly related to dietary linoleate content up to a maximum level between 4 and 5%. Once this level is reached, the amount of fat added to the diet was the determining factor in tumour incidence. In addition, a high dependence of mammary tumours on supplementation with essential fatty acids is evident from *in vivo* and *in vitro* studies (HOPKINS and CARROLL, 1979; KIDWELL et al.,1982). This finding has an impact on epidemiological studies also, as they should include not only a validation of the total amount of fats consumed, but also the proportion of essential fatty acids. Further evidence that the type of fat may be an important determinant of mammary tumorigenesis is indicated by recent studies which show a protective effect of long-chain polyunsaturated fatty acids of the *omega*-3 class. These are found in low amounts in meat fats, but in higher concentrations in fish oils. The protective effects operate at low dose levels (KARMALI et al., 1984) and persist even when the total fat content of the diet is high (OZA and KARMALI, 1986).

Besides specific effects of certain fatty acids the issue of dietary **energy** has to be discussed. Heavier body weight has been directly associated with cancer deaths in men and women (LEW and GARFINKEL, 1979). A higher incidence of colon and rectal cancers is found amongst overweight men, whilst cancers of the reproductive tract, gall bladder and the breast were more commonly observed in overweight women. In particular for hormone-dependent cancers in women, there is evidence for an association to overweight (LA VECCHIA et al., 1982; SIMPSON and MENDELSON, 1987), and an impressive number of case-control studies has demonstrated a relationship between obesity and the risk of breast cancer (HELMRICH et al., 1983). However, the mechanism by which overweight may enhance risk of breast cancer in post-menopausal years, but provide protective effects in premenopausal years is not known (WILLETT et al., 1985). The association between obesity and incidence of hormone-dependent cancers has been attributed to estrogen metabolism, as increased adiposity enhances the capacity to synthesize estrone in adipose tissues, and decreases the sex-hormone-binding globulin (SHBG) capacity, resulting in an increased bioavailability of free estrogens (SIITERI, 1987). In addition, alteration of prostanoid formation including the discussed effects of certain fatty acids seem to contribute to the development of breast cancer (WILLIAMS and DICKERSON, 1987).

The role of dietary **cholesterol** in carcinogenesis has rarely been investigated. However, since cholesterol is a precursor of bile acids, which may act as co-carcinogens, some investigators associate dietary cholesterol to cancer. There is a weak correlation between low cholesterol levels and cancer incidence. As hypocholesterolemia is a general finding in pre-cancerous conditions, it remains unclear whether or not dietary cholesterol plays an active role in tumour development.

Cholesterol levels are mediated by fat intake and the presence of **dietary fibre**. Reviewing epidemiological and case-control studies BINGHAM (1990) could conclude that there is evidence that fibre can be associated to a reduction in health risk. In addition, a consistently protective effect of vegetables reflect the fact that vegetables are a major source of fibres in Western diets, whereas beans and peas were associated with an increased risk (TUYNS et al.,1987). The suggestion that dietary fibre is protective in colorectal cancer is based on the fact that cereal fibres increase faecal weight (stool volume), dilute large intestinal contents and enhance transit time. This mediates the *in situ* concentration of bile acids and reduces the contact time between faecal mutagens and putative cancerogens (present in the diet) and bowel mucosa. In addition, dietary fibre increases the yield of short-chain fatty acids (SCFA) by physiological anaerobic fermentation in the gut, alters N-metabolism and reduces pH values, factors, which are associated to carcinogenesis as well. However, no relationship between dietary fibre and fecapentan excretion can be observed. Fecapentanes are compounds which are produced by the large bowel bacterial flora and have been discussed to be important factors in colon carcinogenesis (SCHIFFMAN, 1987; SCHIFFMAN et al.,1988).

Large bowel cancer incidence ranges in most countries second to lung cancer in men and to breast cancer in women and consistent reports show a strong positive association between the consumption of total fat and death rates from breast and colon cancer (CORREA, 1981), and a negative association between dietary fibre and colon cancer (ARMSTRONG and DOLL, 1975). However, the association between fat intake and cancers of breast and colon as concluded from cross-cultural studies and experimental research have not been consistently supported by case-control studies carried out since 1982 (BYERS, 1988; BERRINO and MUTI, 1989). Therefore, a number of recent critical reviews have addressed the need to develop biochemical markers which reflect the mechanim(s) by which nutrients may influence carcinogenesis.

MUTAGENIC COMPOUNDS IN MEATS

The evaluation of the role of certain diets on the risk for cancer implies the discussion on the impact of food contaminants to health risk. Mutagens, i.e. compounds which induce a mutagenic response in bacterial or somatic cells, are considered to be potential carcinogens since first in 1973 AMES et al. (1990) described the *Salmonella typhimurium* assay as a screening method for carcinogens. In a recent article AMES et al. pointed out that cooking of food is a major dietary source of potential carcinogens as heating produces about 2 grs (per person per day) of mostly untested burnt material containing many carcinogens including polycyclic hydrocarbons, heterocyclic amines and nitrosamines. However, prior to the discussion of mutagenic compounds in meats it has to be stressed that other foods as well contain a high number or mutagenic compounds, which are produced as plant metabolites under physiological conditions (AMES et al.,1990).

Polycyclic Aromatic Hydrocarbons

It was discovered late in the 18th century that products of pyrolysis of organic matter were capable of causing human cancer. However, it was not until the 1930s that polycyclic aromatic hydrocarbons (PAH's) were indentified as causative agents. At least 100 structurally distinct PAH's can be found in the environment and in food samples. PAH's occur in the residue of smoke, tar, soot, coal, tobacco smoke, petroleum, or combustion effluent. In addition, PAH's are also natural products of plant metabolism and some microorganisms. PAH's are not primary carcinogens but require metabolic activation by liver enzymes to form epoxides as the ultimative carcinogens.

PAH's can contaminate meats by various routes (HOWARD and FAZIO, 1980). In particular the charring or cooking by an open flame or direct exposure to an electrical element can result in the formation of PAH's. Smoking of meats or fish may contaminate the product, however, modern technology in industrial smooking considerably reduces the contamination rate (NAGAO et al.,1977; LARSSON et al.,1988; POTTHAST et al.,1988). Thus, the dietary intake of PAH's with meats is determined predominantly by the habits of food preparation in private households. Commercially smoked meats contain low amounts of PAH's ranging between 0.1 - 1.5 µg/kg (measured as 3,4 Benzo(a)pyrene (B(a)P)), whilst in home made smoked hams levels up to 2000 µg/kg have been detected. Certain countries like Germany have set maximum permissible levels for B(a)P allowing final concentrations up to 1 µg/kg B(a)P in finished meats.

From a survey in UK total diets, assessing the individual contribution of nutrients to total intake of PAH's, it was concluded that oils and fat (including butter, cheese and margarine) provide the highest PAH contribution to the total dietary amount, followed by cereals and fruits. As commercially smoked meats and fish have low PAH concentration and are more seldom consumed, these items provide only a minor contribution to the total PAH intake (DENNIS et al.,1983).

N-nitroso compounds

N-nitroso compounds form a group of more than 300 chemically diverse substances and most of them are capable of inducing tumours in animals at a 90% risk (PREUSSMANN and STEWART, 1984). N-nitroso compounds can be devided into two general types based on their chemical structure: the nitrosamines and the nitrosamides. Both classes are potent animal carcinogens but nitrosamides are environmentally unstable, whilst nitrosamines are stable under conditions found in most foods.

The occurrence of N-nitroso compounds in meats, beer, dried products and fish has been reviewed by HARVERY and FAZIO (1985) and HOTCHKISS (1987). Cured meats have been considered to be one of the main routes of nitrite exposure and consequently as a source for preformed nitrosamines and the *de novo* synthesis of N-nitroso compounds. The latter can proceed easily in the gastric juice of a consumer. Consequently, various countries have layed regulations on the permissible amount of nitrate in curing salts, as for example Germany, a country having a long tradition in a variety of cured meats and sausages (LEISTNER, 1981).

Recently, more attention has been devoted to endogenous nitrite formation following the ingestion of nitrates (BOS et al.,1988). After oral administration nitrate is recycled through the saliva into the oral cavity, where it may be converted into nitrite. However, the amount of nitrate-nitrite conversion depends not only on the dietary nitrate intake, but also on an individual capacity for conversion, of which the mechanism is not known. Thus, the amount of endogenous nitrite cannot be compared with the nitrite concentrations present in foods. In addition, nitrosamine formation in gastric juice is katalyzed or inhibited by several food

components like chlorogenic acid, thiocyanate ions, polyphenolic compounds, the vitamins C and E and selective amino acids like proline, hydroxyproline and glycine (LATHIA and KLOEP, 1987).

Heterocyclic Amines (Protein Pyrolysis Products)

Heterocyclic amides comprise a group of compounds formed during the pyrolysis of proteins and amino acids including glutamic acid, phenylalanine, ornithine and soybean globulin. Their occurrence in heated meat and fish was first described by NAGAO et al. (1977), who could demonstrate that extracts of fried beef exert a positive response in the *Salmonella* mutation assay. Mutagen formation during cooking of protein-containing foods appears to be a complex function of food type and composition, cooking methods, time and temperature (PARIZA et al.,1979). The formation of heterocyclic amine mutagens (formed by Maillard reactions) were first considered to proceed only, when heating temperatures exceed 300°C (NAGAO et al.,1983). However, mutagens were formed also during pan frying of salmon and other fish filets at 190°C (KRONE and IWAOKA, 1981). Comparing the mutagenicity of fried and (commercially) canned food KRONE and IWAOKA (1987) found a higher mutagenic activity ratio in fried items. In general, mutagens in heated foods fall into two broad structural categories:

(1) the pyrido-imidazole/indole mutagens (Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, A*a*C, MeA*a*C and EtA*a*C, and
(2) the imidazo-quinoline/quinoxaline mutagens (IQ, MeIQ, MeIQx, 4,8-DiMeIQx and 7,8-DiMeIQx).

Summarizing the results on the mutagenicity of heterocyclic amines it can be recognized that all derivatives are strong mutagens and in particular the IQ-type compounds are more potent than Benzo(a)pyrene, and in TA 98 even more potent than aflatoxin B_1 (for review see FINK-GREMMELS and LEISTNER, 1991).

Results from at least eight heterocyclic amines which have been tested for carcinogenicity in rodent systems indicate tumour induction at a variety of sites both in mice and rats, with the liver being the most common location (SUGIMURA, 1985). The pyrido-imidazole/indole compounds are hepatocarcinogenic when included into the diet of mice (OHGAKI et al.,1984a, SUGIMURA and SATO, 1983). Hepatocarcinomas, squamous cell carcinomas of the zymbal gland, and brain and colon tumours are induced in rats fed Glu-P-1 (TAKAYAMA et al., 1984 a & b). IQ is a strong inducer of unscheduled DNA synthesis in liver cells and is carcinogenic in mice and rats (BARNES et al.,1985; OHGAKI et al.,1984b). MeIQ in the diet induces tumours in the forestomach and livers of mice (OHGAKI et al.,1985).

The total daily *per capita* intake of heterocyclic amines has been estimated to be as high as 100 µg (SIGURA, 1985). Several carcinogenic heterocyclic amines, including Trp-P-1 and Trp-P-2 (MANABE et al.,1987) and MeIQx (YANAGISAWA et al.,1986) and IQ-type amines have been detected in dialysis fluids. Accumulation of Glu-P-1 and Glu-P-2 in plasma of patients with uremia has been described by MURRAY et al. (1989).

The data show that humans are widely exposed to measurable quantities of these compounds (MANABE and WADA, 1988). In addition, the fact that microsomal fractions of human liver can convert MeIQx to potent mutagens in the Ames *Salmonella* assay (MURRAY et al.,1988), and the facts that N-acetylated metabolic products of Glu-P-1 and Glu-P-2 have been found in human urine, bile, liver and kidney (KANAI et al., 1988) indicate that these compounds may be suspect human carcinogens. Thus, the potential human cancer risk exerted by these compounds is hard to evaluate.

Natural occuring compounds and Mycotoxins

There is increasing evidence that the estimate of human risk to cancer is weak, when it is based on selected *in vitro* assays conducted with purified compounds which have been detected to occur in foods. AMES et al. (1990) calculated that 99.99% (by weight) of all natural occurring toxins produced by plants, to protect themselves are mutagenic. One group of toxins which also can be present in meats are mycotoxins, the toxic secondary metabolites of moulds. In the view of the FAO (as presented by DAWSON, 1990) mycotoxins are of global concern and are recognized to be a major contaminant of foods and feeds, mainly staple cereals and oilseeds.

Mycotoxins comprise a structurally diverse familiy of compounds which can induce toxicity in humans and animals. Mycotoxins may reach the consumer by two different ways: (1) The direct route via ingestion of cereals, nuts or fruits and other plant commodities as well as meats which are spoiled by fungi. (2) An indirect exposure is known to occur when toxic residues of mycotoxins persist in meat and other tissues as well as in milk and in eggs from animals, which have been exposed to mycotoxin-contaminated feedstuffs. Mycotoxins which have been detected frequently in food and feed commodities are the aflatoxins, ochratoxins, *Fusarium* toxins, *Pe-*

nicillium toxins and *Claviceps* toxins. Carry-over into milk and edible tisssues have been demonstrated for aflatoxin B_1 (aflatoxin M_1 in milk), ochratoxin A, zearalenone, deoxynivalenol, diacetoxyscirpenol and T-2 toxin (for review see FINK-GREMMELS, 1989, 1990). However, experimental studies indicate that other mycotoxins may occur in meats as well.

Aflatoxin B_1 induces hepatocarcinomas in all animal species tested as well as in humans (PARK and POHLAND, 1986). Ochratoxin A has been discussed to be the causative agent for Balkan endemic nephropathy (BEN) and associated tumours of the urinary system (UTT) (PETKOVA-BOCHROVA et al., 1988). Zearalenone has induced genotoxicity in experimental studies (KUIPER-GOODMAN et al., 1987). Thus, it seems necessary to monitor the presence of mycotoxins in foods to exclude a frequent exposure of consumers.

Mycotoxin contamination of meats might also occur during traditional ripening of hams and raw sausages (LEISTNER, 1984). Therefore, it has to be recommended that mould fermented meats should be produced with the help of selected starter cultures (FINK-GREMMELS et al.,1988). In contrast to cheese ripening where *Penicillium camembertii* and *P.roquefortii* are widely used (PITT et al.,1986), we could demonstrate that selected *P.nalgiovense* isolates can be recommended for Salami-type sausages (LEISTNER, 1990; FINK-GREMMELS, 1990).

RESIDUES IN MEATS

In the last four decades there have been revolutionary developments in synthetic organic chemistry. As of spring 1985 the US Environmental Protection Agency (EPA) had a list of 60 000 marketed chemicals, and new compounds can be expected at a rate of 1200 per year. It is therefore quite obvious, that consumers are worried about the quality of their daily food supplies and the public press worldwide reports about an increasing number of chemical substances which are contaminating our foods and are considered to be a potential health risk. A wide spectrum of undesirable substances can be introduced into foods via a number of routes. In principle they can be catagorized into two groups:

(1) Substances, present as naturally occuring compounds or contaminants, and

(2) Substances, intentionally introduced during the cource of food manufacturing or preparation.

The first group includes natural occurring toxins in plants such as oxalates, glycoalkaloids, cyanogenic glycosides, hemagglutinins, quercitin, goitrogens and pyrrolizidine alkaloids; marine toxins including tetrodotoxin, ciguatoxin, scombroid toxin and shellfish poisons; toxic elements (heavy metals) and persistent environmental pollutants (pesticides, polyhalogenated compounds a.o.). Their overall presence - although in most cases in minute amounts - is unavoidable. To screen the frequency of occurrence and concentration of these compounds in foods, most countries have established monitoring systems, providing information about local, seasonal and accidental risks for unexceptable food contamination. The evaluation of these data is based on tolerance levels set by regulatory bodies (the rational of permissible levels will be discussed in the following chapter). Promissing strategies towards an international, worldwide harmonization of threshold levels have been developed by supranational bodies - including the World Health Organisation - with the aim to provide a successful risk management.

Pork, veal and poultry meats are foods which comprise a comparable low risk to be loaded with contaminants, as the short production period (lifespan) prevents accummulation of xenobiotics in edible tissues of these animals. Dairy cows (and sheep and horses) in feed lots or on pasture may during their lifespan more frequently be exposed to various chemicals including contaminants and drugs to prevent or cure diseases. As xenobiotics can be excreted via milk, not only residues in the carcass but also in milk and dairy products have to be considered. In addition, as milk is widely used in meals for infants, the special sensitivity of this group of consumers has to be taken in account when setting tolerance levels. Laying hens excrete xenobiotics via their eggs. As distribution in the egg yolk can result in an delayed recognition of xenobiotic exposure, eggs should not only be included in total diet analysis but also provide a useful tool in residue monitoring systemes.

The second group of residue forming xenobiotics is assembled by substances which are intentionally introduced into food production. These are agro-chemicals, veterinary drugs and feed additives as well as food additives. As the presence of violative residues from these compounds has to be considered as avoidable, stepwise regulations have been established to exclude that substances which have the potential of a health risk do not enter the production process. In principle, the following steps are involved: (a) a

premarketing approval system, (b) an assessment of the impact of possible residues (inadequate use) on public health, and (c) the development of sensitive qualified methods for analysis in residue monitoring.

Standards and guidelines given by national authorities may vary to a certain extend in different countries. However, there is a principle agreement that substances, exerting serious toxic effects in low concentrations such as teratogenicity, immunosuppression, mutagencity or carcinogenicity are banned before being introduced into the marked. Consequently, the rational for an assessment of the impacts of possible residues - for instance after inadequate use - is soundly based, as unpredictable, non dose-dependent phenomenas such as genotoxicity and carcinogenicity can be excluded already in the first phase of approval. However, an increasing understanding of the biotransformation in target animal species (meat producing animals), resulting in a variety of non-toxic but also toxic derivatives may require a toxicological re-evaluation for certain groups of compounds. In addition, current information and knowledge is insufficient to allow an assessment of the degree of risk based on immunopathological mechanisms (allergic reactions) and resistance induction in bacteria by antibiotics and other antimicrobial compounds.

It is not feasable to present a complete general overview about the incidence of undesirable residues in meat and meat products as long as sensitivity of the methods applied for the detection and sampling frequency vary that considerably in the various countries. In 1986 FSIS (USA) inspected 121 million red meat animals and almost 5 billion birds and supervised the production of 67 billion pounds of processed meat and 60 billion pounds of processed poultry. Also in 1986, violative residues of chlorinated hydrocarbons were detected in 0.5% of swine tested, 0.1% of cattle and calves tested, and in 0,5% of all sheep. None of the chicken or turkey tested gave evidence for an occurrence of these compounds in their meats. In addition, organophosphates were not detected in any species. Antibiotic residues were reported in 1.2% of red meat animals and 0.5% of poultry tested, and sulfonamide residues in 1.6% of poultry tested (*cit.* after PULLEN,1990). The meat inspection programme in the U.S. includes analysis of about 100 different compounds which are suspected to form residues in animal tissues.

In 1988 in Germany (BRD) 0.0038% of all beef carcasses slaughtered, 0.13% of veals and 0.0029% of swine contained detectable residues above the legally defined permissible levels (Statistisches Bundesamt).
These data demonstrate, that - albeit the recognized environmental pollution and the intensity of animal production in these countries - the incidence of residues is not extremely high. In addition, it has to be stressed that the occurrence of a positive finding in residue monitoring does not inevitable imply a health hazard. It simply indicates, that residue concentrations above a given permissible level have been detected.

FOOD TOXICOLOGY AND RISK ASSESSMENT

Risk assessment is a term which is used with increasing frequency from scientists, regulators and consumers and very often risk assessment is misinterpretated and misused for the listing of analytical data obtained during food inspection only. Risk assessment - in the framework of food toxicology - is the biomedical assessment of the possible impact of a compound occurring in foods to public health.

Foods, even those from conventional sources, such as fruits, nuts or meat of game animals can be completely analyzed to be a mixture of hundreds of chemical compounds. Thus, the description of an undesirable compound or residue first requires a definition: *what is natural*. For example, since centuries meats have been predominantly consumed after having been cooked, in old days over an open fire. Modern analytical methods enable us to detect a pleora of highly mutagenic and experimental carcinogenic pyrolysis products (heterocyclic amines) in heated meats. Following the conventional *Zero Risk Concept* in risk assessment this would mean that all heated protein containing foods, including meat, fish and soybean products have to be completely banned. In contrast, if we consider these compounds to be *natural*, and thus providing only an acceptable risk for the consumer, this would imply that we have to use the same tolerance margin of acceptable risk also in assessing industrial chemicals.

Both concepts, the zero risk demand as well as the axiom of natural compounds not being hazardous, provide no serious contribution to a successful risk management in food production. Despite the generalized desire of modern-days society to live in a risk-free world, consumers have to accept that a zero risk is neither achievable nor natural. However, as safety of foods is no longer contemplated as an absolute term, all efforts to define and achieve *the minimum* risk have to be encouraged.

Risk is the measured or estimated probability of an event (injury, disease or death) inherent within our daily activities. Estimation of risk associated with contaminants or residues in foods requires identification of the potential adverse health effects (hazard identification) as well as identification of the amounts consumed and the possible routes by which a person might come into contact with the incriminated substance (exposure assessment). In principle, the overall risk is low for a compound which occurs seldom in foods (i.e. accidental contamination) and has a low, dose-dependent toxicity (FINK-GREMMELS and LEISTNER, 1986).

It is generally accepted that the potential hazard of a substance occurring in foods has to be estimated from all toxicological data available, including acute and chronic toxicity in different animal species, organ specific effects, teratogenicity and embryotoxicity, immunotoxicity, mutagenicity and carcinogenicity. Envisaging risk assessment adverse biological activities can be devided into two categories:

(a) linear dose dependent effects, and
(b) non-linear dose dependent effects.

Dose dependent effects include all pharmacological properties of a compound, i.e. the reversible alteration of physiological functions. For these effects a reliable *no effect level* or *safety level* can be establised. If experimental data on pharmacological properties are only available from studies in animals, the estimation of a safe level for humans accounts **safety factors** to resemble the uncertainties in extrapolating animal data to humans. The size of the safety factor applied may vary, although a common and pragmatic approach is to use the factor of 10 x 10. This factor 100 is based on the assumption that man is 10-fold more sensitive than laboratory animals from which most of the biological data are achieved. This species-specific extrapolation factor is multiplied again by 10 in consideration of qualitative aspects of bioassay data, steepness of the dose-response curve, extend of response and comperative pharmacokinetics and metabolism rates. Additional safety factors have been introduced for selected effects as for instance the factor 500 in relation to teratogenicity if only tested in two animals species. Consequently, the safety factor might be reduced when assessing compounds for which sufficient human data are avaible.
The advances of fixed safety factors are obvious: they are easy to understand, they are applicable for all kinds of compounds which occur in our environment and foods, and they allow a comparative judgement of all undesirable compounds.

For food ingredients or contaminants a fixed safety factor and consequently a one-way equation in the calculation of an acceptable exposure rate for man allows the precise definition of an **acceptable daily intake.** The latter can be used, to assess the analytical data obtained from food surveillance programmes and allows a judicious and simple yes/no answer. The safety factor concept has been used for FAO/WHO decisions and in national regulatory bodies.

Criticism about the safety factor concept arises when in the procedure of toxicological testing of a compound a potential for irreversible toxic effects, i.e. a potential carcinogenicity, becomes evident. For compounds which might interact with DNA and subsequently alter DNA bases with the result of carcinogenesis, the threshold concepts is not accepted. In the view of the multistage concept of chemical carcinogenesis various mathematical models have been developed, based on different assumptions (for review see JOHANNSEN, 1990):

* The *linear models* are based on the assumption that increase of tumours by a xenobiotic augments an already ongoing biological process.
* *Mechanistic models* are considering an age-specific tumour rate, defined as the proportion of people in any specific age group.
* The class of *tolerance distribution models* assume that each member of a population will develop a tumour if exposed to a carcinogen exceeding a critical level.
* *Time-to-tumour models* try to describe a complex relationship between dose and tumour-latency and therefore require a variety of additional toxicological data.
* *Two stage response models* consider transformation probabilities and cellular dynamics (proliferation, taken from mechnistic studies rather than chronic bioassays, to estimate age specific tumour incidence rates.

The mathematically based approaches result in extreme variations (up to 1:1,000 000) of the estimates of a virtually safe dose (i.e. concentration in food).

One of the major criticism towards these mathematically based models is that they neglect the metabolic fate of a xenobiotic in the organism. As it is known that a growing number of chemicals only produce an experimental carcinogenic response after they have undergone (speciesspecific-)metabolic activation, the involvement of non-linear kinetics in the bioactivation process from a procarcinogen to an ultimate carcinogen has to be considered. As a consequence, the concept of **physiologically based pharmacokinetic models (PB-PK)** has been developed. This approach integrates absorption, distribution, biotransformation, excretion and receptor site interactions in the target organism (man) into the extrapolation of high experimental dosages (virtual risk) to manyfold lower levels on human exposure (actual risk) in the interpretation of the phenomenon of tumour induction (HOEL et al.,1983). Thus, the PB-PK concept is the unique model which is not based on external concentrations of a potential hazardous compound (as present in foods) only, but also on the internal (in the organism) active aliquote at the actual site of possible pathological response.

Another item for criticism in risk evaluation is the possible role of substance-interactions (rendez-vous effect), which might have a considerable impact on immunpathological alterations (allergy, idiosynchratic reactions) and the effects of single or groups of residues on the resistance-induction in bacteria. For both possible events a reliable concept for risk assessment has not been established and current knowlegde is based on experimental data and a few epidemiological studies.

SUMMARY AND CONCLUSION

Meat consumption has been suspected to be responsible for a variety of metabolic diseases and cancer. In addition, mutagenic and carcinogenic compounds generated during preservation and cooking of meats are considered to contribute to the hazardous properties of meat as a food. However, in recent years developments in basic and applied sciences gave *no evidence* that simply eliminating meats from our diet would have a significant effect to human health (PHILIPPS et al., 1983). However, greater appreciation of the scientific facts related to meats in our diet - both favourable and detrimental - should stimulate new concepts in animal production and meat technology. Nutritionists seem often to neglect that eating is not only a physical need but also an *art*, contributing to the overall well-being and life-style. Meat technologists trend to overestimate current topics in nutritional science and basic medical research. Thus, a more integrated approach of meat producers, meat scientists, nutritionists, medical personnel and consumers seems to be the only pragmatic recommendation to improve the understanding of the role meat plays in our diet.

REFERENCES

AMES, B.N., LEE, F.D. and DURSTEN, W.E. (1973): An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Natl. Acad. Sci. USA 70:782-786.

AMES, B.N., PROGET, M. and GOLD, L.S. (1990): Dietary pesticides (99,99% all natural). Proc. Natl. Acad. Sci. USA 87:7777-7781.

ARMSTRONG, B. and DOLL, R. (1975): Environmental factors and cancer incidence and mortality in different countries, with special reference in dietary practices. Int. J. Cancer 15:617-631.

BARNES, W.S., LOVELETTE, C.A., TONG, C., WILLIAMS, G.M. and WEISBURGER, J.H. (1985): Genotoxicity of the food mutagen 2-amino-3-methylimidazo(4,5-f)quinoline (IQ) and its analogs. Carcinogenesis 6:441-444.

BEATON, G.H. and CHERY, A. (1988): Protein requirements of infants, a re-examination of concepts and approaches. Am. J. Clin. Nutr. 48:1351-1361.

BERRINO, F. and MUTI, P. (1989): Mediterranean diet and cancer. Europ. J. Clin. Nutrition 43 (Suppl. 2), 49-55.

BINGHAM, S.A. (1990): Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. Proc. Nutr. Soc. 49:153-171.

BLOCK, G., DRESSER, C.M., HARTMAN, A.M. and CARROLL, M.D. (1985): Nutrient sources in the American diet: Quantitative data from the NHANES II survey. II Macronutrients and fat. Am. J. Epidemiol. 122:27-35.

BONAMONE, A. and GRUNDIG, S.M. (1988): Effects of dietary stearic acid on plasma cholesterol and lipoprotein levels. N. Eng. J. Med. *318*:1244-1247.

BOS, P.U.J., VAN DEN BRANDT, P.A., WEDEL, M. and OCKHUIZEN, Th. (1988): The reproducibility of the conversion of nitrate to nitrite in human saliva after a nitrate load. Food. Chem. Toxicol.26:93-97.

BYERS, T. (1988): Diet and cancer. Any progress in the interim ? Cancer 62,1713-1724.

CORREA, P. (1981): Nutrition and cancer: Epidemiologic correlations. In: "The Practice of Cancer. Prevention in Clinical Medicine" (G.R. Newell, ed.). Raven Press, New York. pp. 1-10.

DAWSON, R.J. (1990): Global concern on mycotoxins. Food Control 1:15-16.

DENNIS, M.J., MASSEY, R.C. and McWEENY, D.J.(1983): Analysis of polycyclic aromatic hydrocarbons in UK total diets. Food Chem. Toxicol. 21:569-574.

DOLL, R. (1990): An overview of the epidemiological evidence linking diet and cancer. Proc. Nutr. Soc. 49:119-131.

ERDMAN, J.W. (1989): Nutrition: Past, present and future. Food Technology pp. 220-227.

FAO/WHO/UNU (1985): Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. Technical Report Serien no. 724. World Health Organization, Geneva. 34 pages.

FINK-GREMMELS, J. (1989): Bedeutung der Mykotoxinaufnahme für das Schlachttier. Dtsch. tierärztliche Wschr. 96:360-363.

FINK-GREMMELS. J. (1990): Möglichkeiten und Risiken einer Lebensmittelkontamination durch Mykotoxine. In: "Proceedings of the International IFF-Symposium", held October 30/31 in Braunschweig, Deutschland. pp. 61-86.

FINK-GREMMELS, J. and LEISTNER, L. (1990): Toxicological evaluation of moulds. Food Biotechnol. 4:579-584.

FINK-GREMMELS, J. und LEISTNER, L. (1991): Mutagene in der Nahrung. In: "Ernährungstherapie bei Tumorerkrankungn" (P. SCHAUDER, ed). Karger Verlag, Basel. *in print*

FINK-GREMMELS, J., EL-BANNA, A.A. and LEISTNER, L. (1988): Developing mould starter cultures for meat products. Fleischwirtsch. 68:1292-1294.

GRIESEN, G. (1986): Mild anemia in African school children: Effect on running performance and an intervention. Acta Paediatr. Scand. 75:662-666.

HAMSTER, A. (1988): Apolipoproteins, dyslipoproteinemia and premature coronary heart disease. Acta Med. Scand. 223:389-393.

HARPER, A.E. (1988): Nutrition: From myth and magic to science. Nutr. Today 23:8-12.

HAVERY, D.C. and FAZIO, T. (1985): Human exposure to nitrosamines from foods. Food Technol. 85/180-83.

HELMRICH, S.P., SHAPIRO, S. and ROSENBERG, L. (1983): Risk factors for breast cancer. Amer. J. Epidemiol. 115:241-245.

HOEL, d.J., KAPLAN, N.L. and ANDERSON, M.W. (1983): The implication of non-linear kinetics on risk estimation in carcinogenesis. Science 219:1032-1035.

HOPKINS, G.J. and CARROLL, K.K. (1979): Relationship between amount and type of dietary fat in promotion of mammary carcinogenesis induced by 7,12-dimethylbenz(*a*)anthracene. J. Natl. Cancer Inst. 62:1009-1012.

HOTCHKISS, J.H. (1987): A review of current literature on N-nitroso compounds in foods. Adv. Food Res. 31:54-67.

HOWARD, J.W., FAZIO, T. (1980): Review of polycyclic aromatic hydrocarbons in foods. J. Ass. Off. Anal. Chem. 63:1077-1084.

IP, C. (1987): Fat and essential fatty acid in mammary carcinogenesis. Amer. J. Clin. Nutrition 45:218-224.

JOHANNSEN, F.R. (1990): Risk assessment of carcinogenic and noncarcinogenic chemicals. Critical Rev. Toxicol. 21:341-367.

KANAI, Y., MANABE, S.H. and WADA, O. (1988): *In vitro* and *in vivo* N-acetylation of carcinogenic glutamic acid pyrolysis products in humans. Carcinogenesis 9:2179-2184.

KARMALI, R.A., MARSH, J. and FUCHS, C. (1984): Effects of omega-3 fatty acids on the growth of a rat mammary tumor. J. Natl. Cancer Inst. 73:457-461.

KIDWELL, W.R., KNAZECK, R.A., VONDERHAAR, B.K. and LASONCZY, I (1982): Effects of unsaturated fatty acids on the development and proliferation of normal and neoplastic breast epithelium. In: "Molecular Interactions of Nutrition and Cancer" (M.S. Arnott, J. van Eyes and Y. Yang, eds). Raven Press, New York. pp. 219-226.

KRONE, C.A. and IWAOKA, W.T. (1981): Mutagen formation during the cooking of fish. Cancer Lett. 14:93-99.

KRONE, C.A. and IWAOKA, W.T. (1987): Commercial food processing operations and mutagen formation. J. Food Protect. 50:167-174.

KUBOW. S. (1990): Toxicity of dietary peroxide products. Trends in Food Sci. Technol. 1:67-71.

KUIPER-GOODMAN, T., SCOTT, P.M. and WATANABE, H. (1987): Risk assessment of the mycotoxin zearalenone. Regul. Toxicol. Pharmacol. 7:253-306.

LARSSON, B.K., PYSSALO, H. SAURI, M. (1988): Class separation of mutagenic polycyclic organic material in grilled and smoked food. Z. Lebensm. Unters. Forsch. 187:546-551.

LATHIA, D. and KLOEP, D. (1987): Einfluß von Nahrungsmittelinhalts- und Zusatzstoffen auf die Nitrosaminbildung unter physiologischen Bedingungen - ein kurzer Überblick. Ernährung/Nutrition 11:98-101.

LA VECCHIA, C., FRANCESCHI, S. and GALLUS, G. (1982): Prognostic features of endometrial cancer in oestrogen users and obese women. Amer. J. Obstetrics, Gynecol. 144:387-390.

LEISTNER, L.(1981): Neue Nitrit-Verordnung der Bundesrepublik Deutschland. Fleischwirtsch. 61:338,341-342,344,346.

LEISTNER, L. (1984): Toxinogenic penicillia occurring in feeds and foods: A review. Food Technol. Aust. 36:404-406, 413.

LEISTNER, L. (1990): Mould-fermented foods: Recent development. Food Biotechnology 4:433-441.

LEW, E.A. and GARFINKEL, L. (1979): Variations in mortality by weight among 750.000 men and women. J. Chronic Diseases 32:563-576.

MANABE, S.H., YANAGISAWA, S.B., GUO, S., ABE, S., ISHIKAWA, Y. and WADA, O. (1987): Detection of Trp-P-1 and Trp-P-2, carcinogenic tryptophan pyrolysis products, in dialysis fluid of patients with uremia. Mutat. Res. 179:33-40.

MANABE, S.H. and WADA, O. (1988): Analysis of human plasma as an exposure level monitor for carcinogenic tryptophan pyrolysis products. Mutat. Res. 209:33-38.

McNAMARA, D.J. (1987): Effects of fat-modified diets on cholesterol and lipoprotein metabolism. Ann. Rev. Nutr. 7:273-276.

McNAMARA, D.J. (1990): Relationship between blood and dietary cholesterol. In: "Meat and Health. Advances in Meat Research" vol. VI (A.U. PEARSON and T.R. DUTSON, eds.). Elsevier Appl. Science - London and New York. pp. 63-87.

MILLWARD, D.J., JACKSON, A.A., PRICE, G. and RIVERS, J.P.W. (1989): Human amino acid and protein requirements: Current dilemmas and uncertainties. Nutr. Res. Rev. 2:109-132.

MILLWARD, D.J. and PRICE, G.U. (1990): Maintenance protein requirements: The need for conceptual re-evaluation. Proc. Nutr. Soc. 49:473-487.

MONSEN, E.R. (1988): Iron nutrition and absorption: Dietary factors which impact iron bioavailability. J. Amer. Dietetic Ass. 88:786-790.

MUNRO, H.N. (1985): Historical perspective on protein requirements: Objectives for the future. In: "Nutritional Adaption in Man" (K. BLAXTER and J.C. WATERLOW, eds.). John Libbey, London and Paris. pp. 155-168.

MURRAY, B.P. BOOBIS, A.R., de la TORRE, R., SIGURA, J. and DAVIES, D.S. (1988): Inhibition of dietary mutagen activation by methylxanthines. Biochem. Soc. Transactions. 16:620-621.

MURRAY, S., GOODERHAM, N.J., BOOBIS, A.R. and DAVIES, D.S. (1989): Detection and measurement of MeIQx in human urine after ingestion of a cooked meat meal. Carcinogenesis 10:763-765.

NAGAO, M., HONDA, M., SEINO, Y., YAHAGI, T. and SUGIMURA, T. (1977): Mutagenesis of smoke condensate and the charred surface of fish and meat. Cancer Lett. 2:221.

NAGAO, M., SATO, S., SUGIMURA, T. (1983): Mutagens produced by heating foods. In: "The Mailard reaction in food and nutrition" (G.R.WALLER, M.S.FEATHER, eds.). ACS Symp.Series 215. Amer. Chem. Soci., Washington. pp. 24-37.

The NATIONAL CHOLESTEROL EDUCATION PROGRAM EXPERT PANEL (NCEP) (1988). Report on detection, evaluation, and treatment of high blood cholesterol in adults. Arch. Intern. Med. 148:36-52.

OHGAKI, H., MATSUKURA, N., MARINO, K., KAWACHI,T. SUGIMURA, T. and TAKAYAMA, S. (1984a): Carcinogenicity in mice of mutagenic compounds from glutamic acid and soybean pyrolysates. Carcinogenesis 5:815-819.

OHGAKI, H., KUSAMA, K., MATSUKURA, N., MORINO, K., HASAGAWA, H., SATO, S., TAKAYAMA, S. and SUGIMURA, T. (1984b): Carcinogenicity in mice of a mutagenic compound, 2-amino-3-methylimidazo(4,5-f)quinoline, from broiled sardine, cooked beef and beef extract. Carcinogenesis 5:921-924.

OHGAKI, H., HASAGAWA, H., KATO, T., SUENAGA, M., UBUKATA, M., SATO, S., TAKAYAMA, S. and SUGIMURA, T. (1985): Induction of tumors in the forestomach and liver of mice by feeding 2-amino-3,4-dimethylimidazo(4,5-f)quinoline (MeIQ). Proc. Jpn. Acad. Ser. B 61:137-139.

OZA, R.P. and KARMALI, R.A. (1986): Dietary effects of *omega*-3 fatty acids on the growth of R3230AC mammary tumor. Fed. Proc. 45: 1089 Abstr.

PARIZA, M.W., ASHOOR, S.H., CHU, F.S. et al. (1979): Effects of temperature and time on mutagen formation in pan-fried hamburger. Cancer Lett. 7:63-64.

PARK, D.L. and POHLAND, A.E. (1986): A rational for the control of aflatoxin in animal feeds. In: "Mycotoxins and Phycotoxins" (P.S. STEYN and R. VLEGGAR, eds.). Elsevier Science Publ., Amsterdam, pp. 473-482.

PETKOVA-BOCHAROVA, T., CHERNOZEMSKY, I.N. and CASTEGNARO, M. (1988): Ochratoxin A in human blood in relation to Balkan endemic nephropathy and urinary system tumours in Bulgaria. Food Addit. Contamin. 5:299-301.

PHILIPPS, R.L., SNOWDON, D.A. and BRIN, B.H. (1983): Cancer in vegetarians. In: "Environmental Aspects of Cancer. The Role of Macro and Micro Components of Foods" (E.L. WYNDER, G.A. LEVEILLE, J.H. WEISBURGER and G.E. LIVINGSTONE, eds.). Food and Nutrition Press Inc., Westport,C.T.. pp. 53-72.

PHILIPSON, B.E., ROTHROCK, D.R., CONNER, W.E., HARRIS, W.S. and ILLINGWORTH, D.R. (1985): Reduction of plasma lipids, lipoproteins and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N. Engl. J. Med. 312:1210-1216.

PITT, J.I., CRUICKSHANK, R.H., and LEISTNER, L. (1986): *Penicillium commune, P. camembertii,* the origin of white cheese moulds, and the production of cyclopiazonic acid. Food Mibrobiol. 3:363-371.

POHLAND, A.E. and WOOD, G.E. (1987): Occurrence of mycotoxins in food. In: "Mycotoxins in Food" (P. Krogh, P., ed.). Academic Press, London-New York. pp. 35-64.

POTTHAST, K., EICHNER, R., FISCHER, K. (1988): Neuere Ergebnisse über die Zusammensetzung von Räucherrauch. Fleischwirtsch.68:991-1000, 1350-1356.

PREUSSMANN, R. and STEWART, B.W. (1984): N-nitroso carcinogens In: "Chemical Carcinogens, ACS Monograph" no. 182 (C.E. SEARLE, ed.). Amer. Chem. Soc., Washington D.C. pp. 643-828.

PULLEN, M.M. (1990): Residues. In: "Meat and Health. Advances in Meat Research" vol. VI (A.U. PEARSON and T.R. DUTSON, eds.). Elsevier Appl. Science - London and New York. pp. 135-156.

REEDS, P.J. (1990): Amino acids and protein scoring patterns. Proc. Nutrition Soc. 49:489-497.

REISER, R. and SHORLAND, F.B. (1990): Meat fats and fatty aacids. In: "Meat and Health. Advances in Meat Research" vol. VI (A.U. PEARSON and T.R. DUTSON, eds.). Elsevier Appl. Science - London and New York. pp. 21-62.

ROSS, M.H. and BRAS, G. (1971): Lasting influence of early caloric restriction on prevalence of neoplasms in the rat. J. Natl. Cancer Inst. 47:1095-1113.

SAUBERLICH, H.E. (1990): Vitamin B_6, Vitamin B_{12} and Folate. In: "Meat and Health, Advances in Meat Research", vol. VI (A.M. PEARSON and T.R. DUTSON, eds.). Elseviers Appl. Science, London and New York. pp. 460-495.

SCHACKY, von C. (1987): Prophylaxis of atherosclerosis with marine *omega*-3 fatty acids. Ann. Internal. Med. 107:890-932.

SCHIFFMAN, M.H. (1987): Diet and faecal genotoxicity. Cancer Surveys 6:653-672.

SCHIFFMAN, M.H., TASSEL, R.V., ROBINSON, A., SMITH, L. and DANIEL, J. (1988): Current research on fecal mutagenicity. Am. J. Epidemiol. 127:415-416.

SCHWEINBERG, F. and BÜRKLE, V. (1985): Nitrate: A co-carcinogen. J. Cancer Res. Clin. Oncol. 109:200-202.

SIGURA, T. (1985): Carcinogenicity of mutagenic heterocyclic amines formed during cooking process. Nutrition Res. 150:33-41.

STATISTISCHES BUNDESAMT (1989): Land- und Forstwirtschaft, Fischerei. Reihe 4.3. Fleischuntersuchung. Metzler-Poeschel, Stuttgart, 6-13.

SUGIMURA, T. and SATO, S. (1983): Mutagen-carcinogens in foods. Cancer Res. (Suppl) 43:2415s-2421s.

SUGIMURA, T. (1985): Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. Mutat. Res. 15:33-36.

SIITERI, P.K. (1987): Adipose tissue as a source of hormones. Amer. J. Clinical Nutrition 45:277-282.

SIMPSON, E.R. and MENDELSON, C.R: (1987): Effect of aging and obesity on aromatase activity of human adipose cell. Amer. J. Clinical Nutrition 45:290-295.

SMITH, J.E. (1990): Fat soluble vitamins: Vitamin A, E, D and K. In: "Meat and Health, Advances in Meat Research" vol. VI (A.M. PEARSON and T.R. DUTSON, eds.). Elseviers Appl. Science, London and New York. pp. 401-459.

TAKAYAMA, S., MASUDA, M., MOGAMI, M., OHGAKI, H., SATO, S. and SUGIMURA, T. (1984a): Induction of cancers in the liver, intestine and various other organs of rats by feeding mutagens from glutamic acid pyrolysates. Gann 75:207-213.

TAKAYAMA, S., NAKATSURU, Y., MASUDA, M., OHGAKI, H., SATO, S. and SUGIMURA, T. (1984b): Demonstration of carcinogenicity in F344 rats of 2-amino-3 methylimidazo(4,5-f)quinoline from boiled sardine, fried beef and beef extract. Gann 75:467-470.

TUYNS, A.J., HALTERMAN, U. and KAAKS, R. (1987): Colorectal cancer and the intake of nutrients: A case control study in Belgium. Nutrition and Cancer 10:181-186.

WALLACE, R.B. and ANDERSON, R.A. (1987): Blood lipids, lipid-related measures and the risk of atherosclerotic cardiovascular disease. Epidemiol. Rev. 9:95-114.

WELSCH, C.W. (1987): Enhancement of mammary tumorigenesis by dietary fat: Review of potential mechanisms. Amer.J. Clinical Nutrition 45:192-202.

WILLETT, W.C. (1987): Implication of total energy intake for epidemiological studies of breast and large bowel cancer. Amer. J. Clin. Nutr. 45:354-360.

WILLETT, W.C., BROWNE, M.L. and BAIN, C. (1985): Relative weight and risk of breast cancer among premenopausal women. Amer. J. Epidemiol. 122:731-740.

WILLIAMS, C.M. and DICKERSON, J.W.T. (1987): Dietary, hormones and breast cancer: The cell membrane as a possible site of interaction of these two risk factors. Europ. J. Surgical Oncology 13:89-104.

WINDHAM, C.T., WYSE, B.W. and HANSEN, G. (1990): Thiamin, riboflavin, niacine and panthothenic Acid. In: "Meat and Health, Advances in Meat Research, vol. VI (A.M. PEARSON and T.R. DUTSON, eds.). Elseviers Appl. Science, London and New York. pp.401-459.

WORTHINGTON-ROBERTS, B.S., BRESKIN, M.W. and MONSON, E.R. (1988): Iron status of premenopausal woman in a university community and its relationship to habitual dietary sources of protein. Am. J. Clin. Nutr. 47:275-279.

YANAGISAWA, H., MANABE, S., KITAGAWA, Y., ISHIKAWA, S., NAKAJIMA, K. and WADA, O. (1986): Presence of 2-amino-3,8-dimethylimidazol(4,5-f)quinoxaline (MeIQx) in dialysate from patients with uremia. Biochem. Biophys. Res. Commun. 138:1084-1089.

YOUNG, V.R. (1987): Kinetics of human amino acid metabolism, nutritional implications and some lessons. Am. J. Clin. Nutr. 46:709-725.