ROUTING SLIP
GENERATED BY: HF-40
DATE: APR 15,2003

FDA CONTROL NUMBER: 03 1780 **TRACER #:** **OS #:**

DATE OF CORRESPONDENCE: 04/07/03 **DATE INTO FDA:** 04/15/03

TO MARK MCCLELLAN, FDA - COMMISSIONER

FROM: ANDREW KIMBRELL, CFS, CENTER FOR FOOD SAFETY

SYNOPSIS: SUBMITS COMMENTS ON FOOD ADDITIVE PETITIONS 9M4697, 1M4727, 9M4682, 9M4695, AND 9M4696, REGARDING IRRADIATED FOODS--DOCKET #'S, 99F-5522, 01F-0047, 99F-4372, 99F-5321, 99F-5322; REF: TRAC #'S 02-2250, 03-953, 03-1774.

LEAD OFFICE: HFA-305 **HOME OFFICE:** HF-40

CONTACT/PHONE#: KELLY M MALONE 301-827-4437

COPIES: GENERAL DISTRIBUTION
HF-1 MARK B MCCLELLAN
HF-2 LESTER M CRAWFORD
HF-3 MURRAY M LUMPKIN
HF-10
HF-40 LA JUANA D CALDWELL
HF-40 ANNE B CRAWFORD
HF-40 INDYA G MUNGO
HFS-1 JOSEPH A LEVITT
HFS-200 ALAN M RULIS
HFS-200 LAURA M TARANTINO

COORDINATION:

SIGNATURE REQUIRED

REFERRALS FROM HF-40

ASSIGNED TO		ACTION	DUE DATE
HFA-305	BUTLERJ	NECESSARY ACTION	

THE CENTER FOR FOOD SAFETY

April 7, 2003

Dr. Mark McClellan, Commissioner
U.S. Food and Drug Administration
14-71 Parklawn Building
5600 Fishers Lane
Rockville, MD 20857

Mr. Joseph Levitt
U.S. Food and Drug Administration
CFSAN HFS-001
Harvey W. Wiley Federal Building
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dr. Alan Rulis
U.S. Food and Drug Administration
CFSAN OFAS, HFS-200
Harvey W. Wiley Federal Building
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dr. Laura Tarantino
U.S. Food and Drug Administration
CFSAN OFAS, HFS-200
Harvey W. Wiley Federal Building
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Food Additive Petition 9M4697, Use of ionizing radiation for pre-processed meat and poultry; both raw and pre-processed vegetables, fruits and other agricultural products of plant origin; and certain multi-ingredient food products; Food Additive Petition 1M4727, Use of ionizing radiation for control of foodborne pathogens in crustaceans and processed crustaceans; Food Additive Petition 9M4682, Ionizing radiation for the control of Vibrio and other foodborne pathogens in fresh or frozen molluscan shellfish; Food Additive Petition 9M4695, Use of ionizing radiation to treat unrefrigerated (as well as refrigerated) uncooked meat, meat products, and certain meat food products; and Food Additive Petition 9M4696, Increase the maximum dose of ionizing radiation permitted in the treatment of poultry products

Greetings,

The FDA is considering the five above-referenced food additive petitions to irradiate a much greater portion of the food supply. The Center for Food Safety (CFS), together with Public Citizen, has filed five earlier sets of comments opposing these petitions on grounds of serious safety issues stemming from scientific studies indicating that certain irradiated foods may cause mutagenic, genotoxic, cytotoxic and tumor promoting effects in lab animals as well as in humans.

CFS submits this further comment in **opposition** to the five petitions, including the attached tabbed information, which is incorporated herein by reference. This comment focuses first on the increased risk

03-1780

Main Office: 660 Pennsylvania Ave., S.E., Suite 302, Washington, D.C. 20003 • Phone: 202-547-9359 • Fax: 202-547-9429
California Office: Bldg. 1062, Fort Cronkhite, Sausalito, CA 94965 • Phone: 415-229-9336 • Fax: 415-229-9340
Email: info@centerforfoodsafety.org • www.centerforfoodsafety.org & www.foodsafetynow.org

of coronary heart disease and other health problems due to the doubling of *trans* fat resulting from irradiation of ground beef (a product potentially included in the pending food additive petitions 9M4697 and 9M4695). The next section of the comment is on further toxicity risks, relying primarily on older studies whose finding raised alarms that FDA apparently did not heed, but whose impact is amplified by more recent findings. The last section addresses pre-*trans* fat nutritional issues surrounding irradiated foods, based on thoughtful reviews of the nutrient destruction caused by the technology. None of the eight studies cited below were ever addressed in FDA's "omnibus" 1986 irradiation rule (51 FR 13376) nor in any other of its irradiation approvals.

Study 1. Effects of irradiation on *trans* fatty acids formation in ground beef.[1] This recent research tested the levels of various fats and fatty acids in irradiated ground beef. The key finding, summarized in Table 4 therein, is that irradiation at room temperature approximately doubled the prevalence of *trans* fatty acids, from 4.6 % in unirradiated samples to 8.5 % in samples irradiated at 4.5 kGy, the maximum allowable dose for fresh ground beef under FDA regulations.

Study 2. Dietary intake recommendations for *trans* fatty acids.[2] In a crucial report on *trans* fatty acids issued last year by the National Academies of Sciences, Institute of Medicine (IOM), the coronary heart disease (CHD) risks presented by these substances, which, again, are doubled in quantity when ground beef is irradiated, are as follows (emphasis added; citations omitted):

> *Similar to saturated fatty acids, there is a positive linear trend between trans fatty acid intake and LDL cholesterol concentrations. Some evidence also suggests that trans fatty acids result in lower HDL cholesterol concentrations (Table 6). Hence, the net result is a higher total cholesterol (or LDL cholesterol):HDL cholesterol ratio. This finding, combined with data from prospective cohort studies (Table 6), has lead to the concern that dietary trans fatty acids are more deleterious with respect to coronary heart disease than saturated fatty acids.*

---

[1] Brito, M.S., A.L.C.H. Villavicencio, and J. Mancini-filho. 2002. Effects of irradiation on *trans* fatty acids formation in ground beef. *Radiation Physics and Chemistry* 63: 337-340.

[2] National Academies of Sciences, Institute of Medicine, Panel on Macronutrients. 2002 Letter Report on Dietary Reference Intakes for *Trans* Fatty Acids (July 10), at p. 14: online at: www.iom.edu/iom/iomhome.nsf/Wfiles/TransFattyAcids/$file/TransFattyAcids.pdf . The excerpt attached includes only the pertinent pages on risks, pp. 5-14.

> *Summary - There is a positive linear trend between trans fatty acid intake and total and LDL cholesterol concentration, and therefore increased risk of CHD, thus* ***suggesting a Tolerable Upper Intake Level (UL) of zero****. Because trans fatty acids are unavoidable in ordinary diets, achieving such a UL would require extraordinary changes in patterns of dietary intake. Such extraordinary adjustments may introduce other undesirable effects (e.g., elimination of foods, such as dairy products and meats, that contain trans fatty acids may result in inadequate intakes of protein and certain micronutrients) and unknown and unquantifiable health risks may be introduced by any extreme adjustments in dietary pattern. For these reasons, no UL is proposed.* ***Nevertheless, it is recommended that trans fatty acid consumption be as low as possible while consuming a nutritionally adequate diet.***

As indicated the evidence suggests a Tolerable Upper Intake Level of **zero**. In any event, *trans* fatty acid consumption should be minimized. This IOM recommendation directly contrasts with the pending petitions which would allow more irradiation of ground beef and other foods, thereby significantly increasing *trans* fatty acid consumption.

It is probable, although not shown in published studies yet, that irradiation of other types of food besides ground beef that contain fatty acids also significantly raises their *trans* fat prevalence. At a minimum information regarding possible *trans* fat increases in all of the foods covered must be obtained by FDA before deciding on the pending petitions, and FDA must consider the cumulative macronutritional effect of American consumers eating such foods in a prospective heavily irradiated diet.

The list of other documented non-cholesterol and non-CHD related health problems associated with *trans* fat is surely well-known to FDA as it proposing a new rule to list the fat on food labels. They include that *trans* fat:[3]

- *lowers the amount of cream (volume) in milk from lactating females in all species studied, including humans, thus lowering the overall quality available to the infant;*
- *correlates to low birth weight in human infants;*
- *increases blood insulin levels in humans in response to glucose load, increasing risk for diabetes;*

---

[3] Health problems list from Trans Fatty Acid Fact Sheet, on Trans Fat Info Web http://www.enig.com/0001t1b.html, (last visited March 24, 20003), maintained by a leading fat researcher, Mary G. Enig, Ph.D., F.A.C.N., Director, Nutritional Sciences Division, Enig Associates, Inc., Silver Spring, MD.

- *affects immune response by lowering efficiency of B cell response and increasing proliferation of T cells;*
- *decreases levels of testosterone in male animals, increases level of abnormal sperm, and interferes with gestation in females;*
- *decreases the response of the red blood cell to insulin, thus having a potentially undesirable effect in diabetics;*
- *inhibits the function of membrane-related enzymes such as the delta-6 desaturase, resulting in decreased conversion of, e.g., linoleic acid to arachidonic acid;*
- *causes adverse alterations in the activities of the important enzyme system that metabolizes chemical carcinogens and drugs (medications), i.e., the mixed function oxidase cytochromes P-448/450;*
- *causes alterations in physiological properties of biological membranes including measurements of membrane transport and membrane fluidity;*
- *causes alterations in adipose cell size, cell number, lipid class, and fatty acid composition;*
- *adversely interacts with conversion of plant omega-3 fatty acids to elongated omega-3 tissue fatty acids;*
- *escalates adverse effects of essential fatty acid deficiency; and*
- *increases peroxisomal activity (potentiates free-radical formation).*

To summarize the apparent risks of eating irradiated ground beef that is, for example, grilled are considerable:[4] – **First**, red meat consumption is a well-known risk factor for a myriad of health problems. **Second**, flame grilling coats the beef with polycyclic aromatic hydrocarbons, which are known carcinogens. **Third**, grilling meat creates heterocyclic amines, which are mutagens and carcinogens associated with both respiratory tract cancers (from the smoke) and colon cancer. **Fourth**, as we stated in our last comment of Feb. 26, 2003, irradiated beef contains the unique radiolytic products, 2-alkylcylcobutanones, which are genotoxic in concentration and act as colon tumor promoters if consumed together with known colon carcinogens (as are present in this case). **Fifth**, the doubling of *trans* fat in irradiated compared to non-irradiated ground beef increases the risks of coronary heart disease. **Sixth**, the *trans* fat increase also increases risks of a variety of other health problems, listed above.

These should give FDA pause to reconsider its past approvals for irradiated ground beef as well as other fatty acid-containing foods, in view of the potential cumulative health impacts. Indeed, with such an array of associated risks it appears that irradiated ground beef should be declared unsafe and unwholesome.

---

[4] Again, ground beef is potentially covered by the pending food additive petitions 9M4697 and 9M4695.

Additional concerns apply to other irradiated foods as well, such as potatoes, discussed below.

Study 3. Human study finding elevated hemoglobin.[5] As part of the report of her Ph.D. research, Jaarma assessed the impact of eating irradiated potatoes over 14 weeks on seven hematologically healthy volunteers (4 m and 3f). The report states:

> *An increased concentration of hemoglobin, especially in the first period of the investigation, was observed in all the individuals... [H]emoglobin values were significantly higher during the [feeding] period than before [the feeding period]... An additional comparison of the values before with the values after shows that a small effect still remains.*

While unpublished, the study was from a reputable Swedish university and is one of the very few human studies involving irradiated foods. It was one in which several women were subjects, whereas most of the human study subjects have been male. Further, the results showing elevated hemoglobin had high statistical significance.

Study 4. Pig study finding elevated hemoglobin.[6] Study 3's findings were corroborated by this published study by Jaarma and Bengtsson, in which elevated hemoglobin levels again were found, most markedly in breeding female pigs (sows) fed irradiated potatoes over 18 weeks, compared to a control group (p. 117 therein, Fig. 2). The effect was marked during the sows' pregnancies. According to the authors (p. 123):

---

[5] Jaarma, M. 1967. Studies of chemical and enzymatic changes in potato tubers and some higher plants caused by ionizing radiation, including studies on the wholesomeness of γ-irradiated potato tubers and effects on some carbohydrates *in vitro. Akademisk Avhandling,* Som med tillstand av kungl. Universitetets i Stockholm (Ph.D. dissertation.). This study was not considered in FDA's 1986 omnibus irradiation ruling, nor was it included in FDA's bibliography of irradiation studies that supported it, although FDA in the past has considered unpublished studies in its safety assessments. The study is cited in an important review paper, P.C. Kesavan and M.S. Swaminathan. 1971. Cytotoxic and mutagenic effects of irradiated substrates and food material. *Radiation Botany* 253:-281. The particular section of the Jaarma paper called "Studies on haemotological effects of γ-irradiated potatoes on human volunteers" begins at p. 13.

[6] Jaarma, M, and G. Bengtsson. On the wholesomeness of γ-irradiated potatoes - II. Feeding experiments with pigs. *Nutr. Dieta* 8:109-129

> *The appreciably faster increasing haemoglobin levels, and the higher final concentrations, which were noted for one or several animals in nearly all of the I [irradiated potato diet] groups, is difficult to explain. The phenomenon is not necessarily a coincidence.*

Study 5. Population study indicating human stillbirths associated with elevated hemoglobin.[7] This is a fundamentally important recent Swedish population-based case control study on the implications of high hemoglobin for pregnant women. Reported in the *Journal of the American Medical Association,* the conclusion of nine years of research on the outcomes of the pregnancies of more than 1,400 women was:

> *High hemoglobin concentration at first measurement during antenatal care appears to be associated with increased risk of stillbirth, especially preterm and small-for-gestational-age (SGA) antepartum stillbirths.*

Earlier studies showed a relationship between high maternal hemoglobin levels and low birth weight, as well as a connection between high hemoglobin levels and hindered circulation in the placenta.[8]

If consumption of irradiated potatoes causes elevated hemoglobin levels in people generally, and in pregnant sows, as Jaarma et al. found in Studies 3 and 4, above, then the findings of Study 5 indicate that consumption of irradiated potatoes by pregnant women would be a risk factor for stillbirths. Particularly in its review of the sweeping FAP 9M4697, which includes use of ionizing radiation for both raw and pre-processed vegetables, fruits, and other agricultural products of plant origin, and multi-ingredient food products, FDA must ensure that it does not permit an easily avoidable national tragedy to occur in the form of an elevated rate of U.S. stillbirths. An array of additional hemoglobin testing for consumption of potatoes and the many other foods that may be irradiated under that petition must be required first. The needed research should emphasize potential effects on pregnant mammals, although it should not be limited to reproductive effects as elevated hemoglobin also can cause an array of other problems, such as blood thickening, bone marrow dysfunction, increasing numbers of clot-forming platelets, and an enlarged liver or spleen.[9]

---

[7] Stephansson, O., P.W. Dickman, A. Johansson, and S. Cnattingius. 2000. Maternal hemoglobin concentration during pregnancy and risk of stillbirth. *JAMA* 284:2611-2617.

[8] *Acta Obstetrica Gynecologica Scandinavica.* 1990. 69:127-133.

[9] See the Merck Manual on hemoglobin, online at www.merck.com .

The connection between human stillbirths and elevated hemoglobin was not medically established when FDA conducted its earlier reviews of irradiation, but FDA must not overlook it now. Three mice and rat studies enclosed with our May 16, 2001, comments on these pending irradiation petitions did find elevated rates of stillbirths and other pregnancy failures in lab animals that ate irradiated diets, such as the Bugyaki et al. study.[10] However, hemoglobin levels were not fully assessed in those studies, thus any contribution such levels may have made to the observed stillbirths remains unanalyzed.[11]

Study 6. OECD study on genetic effects produced by irradiated food.[12] This was an early and useful report of available data on genetic effects (mutations and chromosome aberrations) in various organisms after ingesting irradiated foods. The sections of the report on mammals (pp. 7-11), that document positive genetic effect findings never have been adequately addressed by FDA. Effects in mice and rats associated with irradiated diets include cell aberrations, lymphopenia, and dominant lethal mutations. Several of these mutagenicity findings were confirmed in later publications cited in our May 16, 2001, comments on the five pending petitions.[13]

The OECD study concluded (pp. 15-16) that while certainty regarding likely effects of irradiated food on humans was elusive based on the data then available:

> *Hitherto available data indicate, however, that increased rates of mutation and chromosomal aberrations will probably be induced in certain cases. Although*

---

[10] Study 2 in the May 16, 2001, comment: Bugyaki, L., A.R. Deschreider, J. Moutschen, M. Moutschen-Dahmen, A. Thijs, and A. Lafontaine. 1968. Do irradiated foodstuffs have a radiomimetic effect? II. Trials with mice fed wheat meal irradiated at 5 Mrad. *Atompraxis* 14:112-118; see also Study 3, therein: Moutschen-Dahmen, M., Moutschen J., and L. Ehrenberg. 1970. Pre-implantation death of mouse eggs caused by irradiated food. *International Journal of Radiation Biology* 18:201-216, and Study 12, Vijayalaxmi and K.V. Rao. 1976. Dominant lethal mutations in rats fed on irradiated wheat. *Int. J. Radiat. Biol.* 29:93-98.

[11] The extent to which mice and rat hemoglobin levels are indicative of correlations in humans is unclear, whereas pigs likely are a better indicator for humans.

[12] Organization for Economic Cooperation and Development (OECD), Steering Committee for Nuclear Energy, Study Group on Food Irradiation. 1965. Genetic effects produced by irradiated food and food components. SEN/IR (65)15. Unpublished report by G.T. Scarascia-Mugnozza, A.T. Najaran, and L. Ehrenberg. Paris, France. This is the best copy available.

[13] See, e.g., the positive studies cited in footnote 10, above.

> *experiments indicate that the genetical effect, in cases where it is induced, is relatively small compared to the effect of direct exposure of animals to radiation, the same experiments indicate that the possible effect will not be negligible.*

Rather than being refuted by subsequent evidence, the OECD's statement regarding likely induction of mutations and chromosomal aberrations has been confirmed in many studies, cited in this and our earlier comments.

We now turn back to (pre-*trans* fat) nutritional issues surrounding irradiated foods, based on thoughtful reviews of the nutrient destruction caused by the technology. The reviews were carried out several years ago, reported in two book chapters attached hereto, but FDA has never addressed them.

Study 7. Nutrition chapter of Food Irradiation - Who Wants It?[14] This 1986 review focuses on reported vitamin losses and concludes they are significant. Key quotes that are particularly relevant as FDA considers the sweeping "ready to eat" petition (FAP 9M4697) and its implications for many types of food that together amount to approximately one-third of the typical American diet are:

> *Losses of 20 to 80% are not uncommon and there are still many gaps in the available scientific data....No studies anywhere have assessed whether there will be a significant impact either on the population as a whole or on vulnerable groups within the population.* (p. 51)

> *The food can thus undergo initial losses on irradiation, accelerated losses during storage, and additional losses because of longer storage times, and then lose further vitamins in cooking.* (p. 52)

The authors noted that the common irradiation industry argument that impacts on population-wide dietary sufficiency would be unlikely because people were not expected to eat much of the food "is dangerously close to saying that irradiated food is all right as long as you don't eat it!" In considering FAP 9M4697, FDA no longer has the luxury of making that assumption.

---

[14] Webb, T., T. Lang, and K. Tucker. 1987. *Food Irradiation - Who Wants It?* Thorsons Publishing, Wellingborough, England. Chap. 4. Wholesomeness of irradiated food.

Study 8. Nutrition chapter of Biology of Food Irradiation.[15] The attachment is the bulk of a chapter from the important 1990 synthesis of the irradiation studies by food chemist D.R. Murray. He states his case succinctly:

> *Disproportionate and selective losses of essential nutrients occur in foods as a consequence of irradiation.* (p. 78)

The rest of the chapter supports this in a *tour de force* analysis of negative impacts on fatty acids, vitamins, amino acids, carbohydrates and other essential components, including in combination with cooking, that FDA must address. In considering the evidence as FDA assesses the foreseeable nutritional impacts posed by, in particular, the sweeping "ready to eat" food additive petition, we request the agency to respond to the following questions:

- 1. What would be the impacts of irradiation as proposed in the petition on each important vitamin and other nutritional component in each different food type that is included?

- 2. What would be the projected national rates of consumption of each different food type included in the petition after foreseeable market penetration of the product, e.g., after 5-10 years of marketing?

- 3. How would this projected future consumption vary across age, ethnic, gender, economic status, education status, and other variables in the American population?

- 4. To what extent would the various population groups likely be affected by the nutritional/vitamin impacts identified under question 1., above?

In conclusion, neither you nor American consumers can be reassured that irradiated food is safe and wholesome in view of the combined evidence indicating health and nutrition impacts, as detailed in this and in our previous five sets of comments on the pending petitions. FDA simply cannot ignore scientific papers showing doubled *trans* fat, elevated hemoglobin, mutagenicity, nutrient destruction, and other harms. Public hearings are needed to address the health and nutrition issues we have raised, which could affect tens of millions of consumers, many unknowingly, in particular the potential impacts on pregnant women,

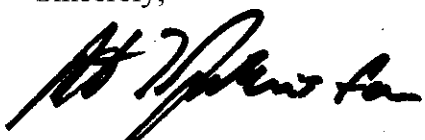
---

[15] Murray, D.R. 1990. *Biology of Food Irradiation.* Research Studies Press Ltd. Staunton, UK. Chap. 4. Radiolytic products and selective destruction of nutrients. Note: pp. 72-78 are omitted. Unfortunately, the appended copy includes some underlining, but the book is out of print and this is the best version available.

children, and other vulnerable populations.

Thank you for your consideration of this comment in opposition to the above-referenced food additive petitions. We also request to meet with you personally on this matter. To arrange a meeting please contact Peter T. Jenkins, Policy Analyst; tel: 202.547.9359 x13; email: peterjenkins@icta.org.

Sincerely,



Andrew Kimbrell, Director
Center for Food Safety
660 Pennsylvania Ave., S.E.
Suite 302
Washington, DC 20003

Attachments (8 tabbed studies)

cc: FDA Food Additive Petition Docket No.s: 99F-5522; 01F-0047; 99F-4372; 99F-5321; 99F-5322 (with attachments)

PERGAMON

Radiation Physics and Chemistry 63 (2002) 337–340

Radiation Physics and Chemistry

www.elsevier.com/locate/radphyschem

Effects of irradiation on *trans* fatty acids formation in ground beef

Mônica S. Brito[a], Anna Lúcia C.H. Villavicencio[a,*], Jorge Mancini-filho[b]

[a] *IPEN-CNEN/SP, Departamento de Aplicações Industriais-Travessa R. no. 400. Cidade Universitária, CEP 05508-910, São Paulo, Brazil*

[b] *FCF/USP, Departamento de Alimentos e Nutrição Experimental-Av. Prof. Lineu Prestes, 580-bloco 14, Cidade Universitária, CEP 05508-900 São Paulo, Brazil*

Abstract

In order to give the consumer the assurance that meat processed by irradiation is a safe product, a great deal of research has been developed in the world. The effect of irradiation on the hygienic quality of meat and meat products is considered as related to the control of meat-borne parasites of humans; elimination of pathogens from fresh meat and poultry; and elimination of pathogens from processed meat. Lipid oxidation and associated changes are the major causes of the quality deterioration of meat during storage. Irradiation of lipids induces the production of free radicals, which react with oxygen, leading to the formation of carbonyls, responsible for alterations in food nutritional and sensorial characteristics. *Trans* fatty acids are present in ground beef and can also be formed during its processing. Interestingly, the *trans* fatty acids, due to their chemical and physical characteristics, show more resistance to the oxidizing process. This property motivated us to investigate the level of the *trans* fatty acids, as well as the level of oxidation in irradiated ground beef. Irradiation of ground beef was performed by gamma rays from a ^{60}Co source. The applied radiation doses were 0; 1.0; 2.0; 3.0; 4.0; 5.0; 6.0; 7.0 and 8.0 kGy. Lipid peroxidation in terms of TBA number and carbonyl content was monitored during storage. The sample characteristics and *trans* fatty acids composition were measured, following irradiation and after 60 and 90 days of storage at −10°C. © 2002 Published by Elsevier Science Ltd.

Keywords: Gamma-radiation; Lipids; Ground meat; Irradiated food

1. Introduction

Among the existing technologies for food preservation, irradiation of food is recognized as a safe and effective method for a range of specific applications. Ionizing radiation uses the high energy of gamma rays or accelerated electrons, thereby ionizing molecules. The use of this treatment on fresh meat could extend shelf life and protect the host against pathogenic bacteria. On the other hand, irradiation treatment brings about some biochemical changes that could affect the nutritional adequacy of food (Giroux and Lacroix, 1998).

Typical composition of ground beef is about 18% lipids and its fatty acids content is divided into 46% saturated, 51% mono-unsaturated and 3% poly-unsaturated (Johnson et al., 1994).

Some of the fatty acids found in meat play important roles in metabolism. Recent interest in *trans* fatty acids (TFAs) was sparked off by epidemiological evidence linking *trans* fatty acids to higher plasma total cholesterol and low-density-lipoprotein (LDL) cholesterol and increased the incidence of coronary heart disease (CHD) (Fritsche et al., 1988).

*Corresponding author. Fax: +55-11-3816-9186.

E-mail addresses: msavoy@net.ipen.br (M.S. Brito), villavic@net.ipen.br (A.L.C.H. Villavicencio).

0969-806X/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0969-806X(01)00647-8

The present study investigated the level of the *trans* fatty acids, as well as the level of oxidation, in irradiated ground beef.

2. Experimental

2.1. Samples

Samples of ground beef were purchased locally in São Paulo, Brazil.

2.2. Irradiation

Irradiation took place in a ^{60}Co Gammacell 220 (A.E.C.L.) installed in Instituto de Pesquisas Energéticas e Nucleares (IPEN)- São Paulo, Brazil. The applied radiation doses were 0, 1, 2, 3, 4, 5, 6, 7 and 8 kGy with a dose rate of 5.8 kGy/h. The samples were irradiated at 25°C (room temperature).

2.3. Lipid analysis

After irradiation the fatty acids of ground beef were extracted according to Folch et al., 1957. The extraction was done after 60 and 90 days of storage, in order to verify the effects of storage time upon the fatty acids level.

2.4. Trans fatty acids analysis

The fatty acid composition was determinated on the lipid extracts after methylation with sulfuric acid and ammonium chloride (Hartman and Lago, 1973). The fatty acid methyl esters (FAME) were analyzed using a CG-500 chromatograph, equipped with fused silica capillary column SP-2560 (100 m, 0.25 mm) and flame ionization detector (FID). Nitrogen was used as the carrier gas. Thermal gradient ranged from 150 to 230°C at 6°C/min. Injector and FID temperature was 250°C. Heptadecanoic acid (C17, Sigma) was added to all samples as an internal standard before the preparation of FAME.

2.5. Statistical analysis

Statistical analysis was developed using a GraphPad Instat, 2.01 version, GraphPad Software.

3. Results and discussion

The major components of lipids from ground beef usually are triacylglycerides and glycerolphospholipids (phospholipids) that may be accompanied by sterols and their esters, fatty acids, sphingolipids, hydrocarbons, etc. Fatty acids range from C12 to C24 in chain length. C16 palmitic acid usually is the major saturated fatty acid, and oleic C18:1 and linoleic C18:2 are the major unsaturated fatty acids.

The fatty acids composition after different storage time for each irradiation doses is presented in Tables 1–3. The main *trans* fatty acid group in ground beef is 18:1. The above results for 18:2 support the observation of Scholfield et al. (1963), that the irradiation observation of the *trans* bond in a poly-unsaturated system is suppressed by the other *cis* and *trans* ethylenic bonds in the molecule.

Table 1
Fatty acid composition of non-irradiated ground beef

Fatty acids	0 days	60 days	90 days
14:00	2.53±0.19	2.34±0.19	2.53±0.19
16:00	25.34±0.09	25.2±0.09	25.18±0.09
18:00	7.28±0.10	7.24±0.10	7.44±0.10
Saturated fatty acid	35.15±0.21	34.78±0.21	35.15±0.21
16:01	1.91±0.04	1.88±0.04	1.82±0.04
18:1 n-9	37.07±0.36	36.77±0.36	36.34±0.36
18:2 n-6	23.09±0.56	24.06±0.56	24.08±0.56
18:3 n-3	0.95±0.03	0.92±0.03	0.89±0.03
20:3 n-6	0.53±0.02	0.52±0.02	0.49±0.02
20:4 n-6	0.62±0.02	0.58±0.02	0.57±0.02
22:5 n-6	0.39±0.05	0.31±0.05	0.31±0.05
22:6 n-3	0.29±0.03	0.29±0.03	0.35±0.03
Unsaturated fatty acid	64.85±0.28	65.33±0.28	64.85±0.28

Table 2
Fatty acid composition of ground beef at 4 kGy

fatty acids	0 days	60 days	90 days
14:00	2.43±0.09	2.34±0.09	2.53±0.09
16:00	25.34±0.08	25.2±0.08	25.18±0.08
18:00	7.68±0.22	7.24±0.22	7.44±0.22
Saturated fatty acid	35.45 ±0.33	34.78±0.33	35.15±0.33
16:01	1.98±0.35	1.98±0.35	1.92±0.35
18:1 n-9	35.57±0.40	34.77±0.40	35.04±0.40
18:2 n-6	20.29±0.13	20.06±0.13	20.08±0.13
18:3 n-3	0.99±0.00	0.99±0.00	0.98±0.00
20:3 n-6	0.57±0.04	0.52±0.04	0.49±0.04
20:4 n-6	0.75±0.10	0.58±0.10	0.57±0.10
22:5 n-6	0.29±0.02	0.31±0.02	0.27±0.02
22:6 n-3	0.19±0.02	0.19±0.02	0.15±0.02
Others	3.92±0.99	5.82±0.99	5.35±0.99
Unsaturated fatty acid	64.55±0.33	65.22±0.33	64.85±0.33

Table 3
Fatty acid composition of ground beef at 8 kGy

Fatty acids	0 days	60 days	90 days
14:00	2.63±0.15	2.34±0.15	2.53±0.15
16:00	25.34±0.18	25.2±0.18	24.98±0.18
18:00	7.68±0.22	7.24±0.22	7.44±0.22
Saturated fatty acid	35.65±0.46	34.78±0.46	34.95±0.46
16:01	1.88±0.03	1.88±0.03	1.82±0.03
18:1 n-9	31.57±0.63	30.77±0.63	30.31±0.63
18:2 n-6	18.29±0.12	18.06±0.12	18.08±0.12
18:3 n-3	0.95±0.03	0.92±0.03	0.89±0.03
20:3 n-6	0.57±0.04	0.52±0.04	0.49±0.04
20:4 n-6	0.75±0.10	0.58 ± 0.10	0.57±0.10
22:5 n-6	0.69±0.21	0.31±0.21	0.31±0.21
Others	9.65±1.69	12.18±1.69	12.85±1.69
Unsaturated fatty acid	64.35±0.53	65.22±0.53	65.32±0.53

There is a decrease of fatty acid and an increase of *trans* fatty acid which occur due to a change in molecular structure of fatty acid, breaking down double bonds, forming free radical and *trans* fatty acids.

The results showed an increase in *trans* fatty acids related to the increase on irradiation dose in ground beef. These results also showed that irradiation induces the *trans* fatty acids formation (Table 4).

Irradiation is a physical method of food processing, consisting of exposing food to ionizing radiation during a limited period of time (Olszyna-Marzys, 1998). The autoxidative process induced by ionizing irradiation in fat is the same as that which occurs in other food processing. However, with irradiation, it is quite accelerated (ICGFI, 1992).

Low irradiation doses (<10 kGy) can kill at least 99.9% of Salmonella in meats and even higher percentage of *Escherichia Coli* 0157:H7 (Oslon, 1998). But irradiation is known to generate hydroxyl radicals that can initiate chain reactions of lipid oxidations in aqueous and oil emulsion systems. Irradiation could produce a large amount of hydroxyl radicals in meat because over 75% of muscle cells are composed of water (Thakur and Sigh, 1995). Irradiation was conducted on fresh-trimmed meat, which incorporated a minimum of fat. It is known that autoxidation of unsaturated fats

Table 4
Levels of trans fatty acids at different radiation doses, analyzed after irradiation after 60 and 90 days of storage at −10°C

DOSE (kGy)	% Trans fatty acids		
	Storage time (days)		
	0	60	90
0	4.60±0.31	4.40±0.31	5.00±0.31
1	8.00±0.00	8.00±0.00	8.00±0.00
2	8.50±0.25	8.30±0.25	8.00±0.25
3	8.00±0.00	8.00±0.00	8.00±0.00
4	8.50±0.29	8.00±0.29	8.50±0.29
5	8.50±0.29	8.50±0.29	8.00±0.29
6	9.50±0.12	9.30±0.12	9.50±0.12
7	9.50±0.00	9.50±0.00	9.50±0.00
8	11.00±0.50	10.50±0.50	10.00±0.50

does not normally take place in animal cells, because it is kept in check by the inhibitory antioxidants (Hampson, 1996).

Irradiation continues to offer a well-known and very useful method of producing radicals and of studying their important reactions for biology, both in vitro and in vivo (Pryor, 1978). The results show that although there is an increase of *trans* fatty acids there is also a decrease in linoleic acid due to oxidation of lipid (Tables 1–3).

The fatty acids are quite susceptible to oxidative process, because the hydrogen of carbons adjacent to double bonds can be replaced by radical species with higher reactivity or through energetic activation like heat, luminosity and ionizing radiation, that induced the production of *trans* fatty acids.

4. Conclusions

Although the gamma radiation has been an excellent method to conserve meat, the molecular structure of this meat can be changed, and this fact is observed in this paper. The total *trans* fatty acids in non-irradiated ground beef is smaller than the irradiated one. The extraction done in samples irradiated after 90 days of storage is similar to the extraction done at the day of storage.

The increase of *trans* fatty acids in the irradiated ground beef is one of the important factors that can be considered in the irradiation process.

References

Folch, J., Less, M., Sloanne Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Fritsche, J., Steinhart, H., Mossoba, M., Yurawecz, M.P., Sehat, N., Ku, Y., 1988. Rapid determination of *trans* fatty acids in human adipose tissue. J. Chromatogr. B: Biomed. Sci. Appl. 705 (2), 177–182.

Giroux, M., Lacroix, M., 1998. Nutritional adequacy of irradiated meat. Food Res. Int. 31 (4), 257–264.

Hampson, J.W., 1996. Effect of low dose gamma radiation on lipids in five different meats. Meat Sci. 42 (3), 271–276.

Hartman, L., Lago, B.C.A., 1973. Rapid preparation of fatty, methyl esters from lipids. Lab. Pract. 22, 457–477.

ICGFI Document 14. Training manual on operation of food irradiation facilities, 4th Edition. FAO/IAEA/WHO, pp. 1–32.

Johnson, L.P., Williams, S.R., Neel, S.W., Reagen, J.O., 1994. Food service industry market profile study: nutritional objective textural profile of food service ground beef. J. Anim. Sci. 72, 1487–1491.

Olszyna-Marzys, A.E., 1998. Radioactivity and food preservation. Nutr. Ver. 50, 162–165.

Oslon, D.G., 1998. Irradiation of food. Food Technol. 52, 56–62.

Pryor, W.A., 1978. The formation of free radicals and the consequences of the reaction in vivo. Photochem. Photobiol. 28, 787–801.

Scholfield, C.R., Jones, R.O., Butterfield, Dutton, H.J., 1963. Anal. Chem. 35, 1588.

Thakur, B.R., Sigh, R.K., 1995. Combination process in food irradiation. Sci. Technol. 6 (1), 7–11.

Letter Report on Dietary Reference Intakes for *Trans* Fatty Acids

Drawn from the Report on

Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids

A Report of the
Panel on Macronutrients,
Subcommittees on Upper Reference Levels of Nutrients
and on Interpretation and Uses of Dietary Reference Intakes, and the
Standing Committee on the Scientific Evaluation
of Dietary Reference Intakes

Food and Nutrition Board

INSTITUTE OF MEDICINE

TABLE 1 *Trans* Fatty Acid Content in Term Human Milk of Women in the United States and Canada

Reference	Study Population/Stage of Lactation[a]	*Trans* Fatty Acid	Content in Human Milk	
			% of Total Fatty Acids	% of Total Energy[b]
Gibson and Kneebone, 1981	120 women, 40–45 d pp	16:1	Trace	Trace
		18:1	~10	~5.46
Chappell et al., 1985	7 women, 1–37 d pp	18:1(9)	2.6 ± 0.4	1.42
		18:1(7)	0.1 ± 0.03	0.05
		18:1(5)	0.1 ± 0.04	0.05
		18:2(6) c,t + t,c[c]	0.1 ± 0.4	0.05
		Total	2.9	1.57
Chen et al., 1995a	198 samples, 3–4 weeks pp	Total *trans*	7.19 ± 3.03	3.92
Innis and King, 1999	103 women, 2 mo pp	Total *trans*	7.1 ± 0.32	3.88

[a] pp = postpartum.
[b] Calculated using the following values: 40g fat/L milk, 8.87 kcal/g fat, 650 kcal/L milk.
[c] c,t + t,c = *cis, trans* and *trans, cis.*

Early reports suggested a wide range of *trans* fatty acid intakes, 2.6 to 12.8 g/day (Emken, 1995). The lower estimated intakes tended to be derived from food frequency data, whereas the higher estimated intakes tended to be derived from food availability data. More recent data from food frequency questionnaires collected in the United States suggest average *trans* fatty acid intakes of 1.5 to 2.2 percent of energy (Ascherio et al., 1994; Hu et al., 1997) or 5.2 percent of total dietary fat (Lemaitre et al., 1998). Intakes of about 1 to 2 percent of energy have been reported for women in Canada, although the range of intake was wide (Elias and Innis, 2001, 2002). Most recently, *trans* fatty acid intake was estimated using data from the Continuing Survey of Food Intakes by Individuals (Allison et al., 1999). The mean *trans* fatty acid intake for the U.S. population aged 3 years and older was 2.6 percent of total energy intake.

ADVERSE EFFECTS OF *TRANS* FATTY ACIDS

Hazard Identification

Total and Low-Density Lipoprotein Cholesterol Concentrations

Prior to 1980 there was generally little concern about the trend toward increased consumption of hydrogenated fat in the U.S. diet, especially when the hydrogenated fats displaced fats relatively high in saturated fatty acids (Denke, 1995). During the early 1980s studies showed a hypercholesterolemic effect of *trans* fatty acids in rabbits (Kritchevsky, 1982; Ruttenberg et al., 1983). Renewed interest in the topic of hydrogenated fat in human diets, or more precisely *trans* fatty acid intake, started in the early 1990s. The availability of a methodology able to distinguish the responses of individual lipoprotein classes to dietary

modification expanded the depth to which the topic could be readdressed. A report from the Netherlands suggested that a diet enriched in elaidic acid (a subfraction of 18:1 *trans*), compared to one enriched in oleic acid (18:1 *cis*), increased total and low-density lipoprotein (LDL) cholesterol concentrations and decreased high-density lipoprotein (HDL) cholesterol concentration, hence resulting in a less favorable total cholesterol/HDL cholesterol ratio (Mensink and Katan, 1990). Consumption of a diet enriched with saturated fatty acids resulted in LDL cholesterol concentrations similar to those observed after subjects consumed a diet high in elaidic acid, but HDL cholesterol concentrations were similar to those observed after subjects consumed the diet high in oleic acid. A number of studies on the topic have been published since then and have reported that hydrogenated fat/*trans* fatty acid consumption increases LDL cholesterol concentrations (Aro et al., 1997; Judd et al., 1994, 1998; Louheranta et al., 1999; Müller et al., 1998; Sundram et al., 1997) (Tables 2, 3, and 4). Recent data have demonstrated a dose-dependent relationship between *trans* fatty acid intake and the LDL:HDL ratio and when combining a number of studies, the magnitude of this effect is greater for *trans* fatty acids compared to saturated fatty acids (Figure 1) (Ascherio et al., 1999).

Similar to the metabolic/clinical trial data, studies in free-living subjects asked to substitute hydrogenated fat for other fat in their habitual diet resulted in higher concentrations of total and LDL cholesterol (Table 4) (Nestel et al., 1992b; Noakes and Clifton, 1998; Seppänen-Laakso et al., 1993).

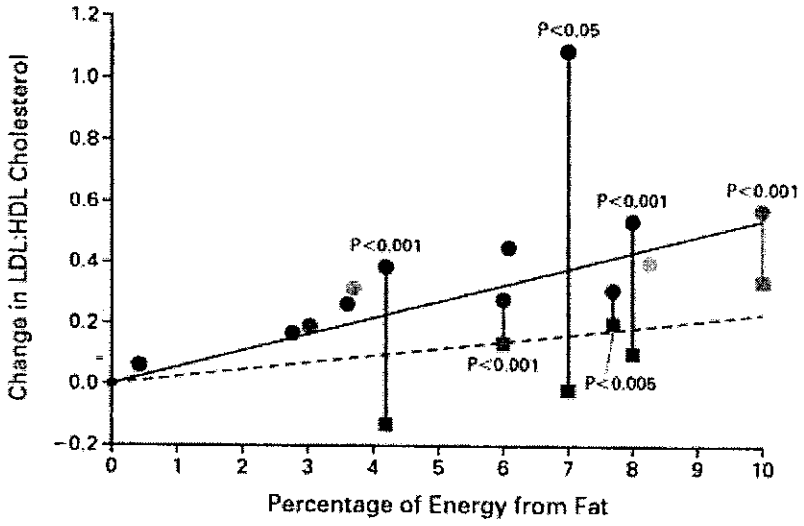


FIGURE 1 Change in the low-density lipoprotein (LDL):high-density lipoprotein (HDL) cholesterol concentration with increasing energy intake from saturated and *trans* fatty acids. Solid line represents the best-fit regression for *trans* fatty acids. Dotted line represents the best-fit regression for saturated fatty acids. Reprinted, with permission, from Ascherio et al. (1999). Copyright 1999 by the Massachusetts Medical Society.

TABLE 2 Dietary *Trans* Fatty Acids (TFA) and Blood Lipid Concentration: Controlled Feeding Trials

Reference	Study Population	Diet[a]	TFA (% of energy)	Blood Lipid Concentrations[b]		
				LDL-C (mmol/L)	HDL-C (mmol/L)	Lp(a) (mg/L)
Mensink and Katan, 1990; Mensink et al., 1992	79 men and women, avg 25–26 y	3-wk crossover, 40% fat				
		10% 18:1	0	2.67[c]	1.42[c]	32[c]
		10% SFA	1.8	3.14[d]	1.42[c]	26[d]
		10% TFA	10.9	3.04[e]	1.25[d]	45[e]
Zock and Katan, 1992	56 healthy men and women	3 wk crossover, 41% fat				
		18:2	0.1	2.83[c]	1.47[c]	
		18:0	0.3	3.00[d]	1.41[d]	
		TFA	7.7	3.07[d]	1.37[d]	
Judd et al., 1994	58 men and women	6-wk crossover, 40% fat				
		18:1	0.7	3.34[c]	1.42[c]	
		SFA	0.7	3.64[d]	1.40[c,d]	
		moderate TFA	3.8	3.54[e]	1.47[e]	
		high TFA	6.6	3.60[d,e]	1.38[d]	
Aro et al., 1997	80 healthy men and women, 20–52 y	5-wk intervention, 33% fat				
		18:0	0.4	2.89[c]	1.42[c]	270[c]
		TFA	8.7	3.13[d]	1.22[d]	308[d]
Sundram et al., 1997	27 men and women, 19–39 y	4-wk crossover, 31% fat				
		18:1	0	3.17	1.25	128.3
		16:0	0	3.15	1.26	122.0
		12:0 + 14:0	0	3.57	1.18	134.3
		TFA	6.9	3.81	1.05	153.3
Louheranta et al., 1999	14 healthy women, avg 23 y	4-wk crossover, 37% fat				
		18:1	0	2.53	1.37	225 (units/L)
		TFA	5.1	2.64	1.31	220 (units/L)

[a] SFA = saturated fatty acid.
[b] LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, Lp(a) = lipoprotein(a).
[c,d,e] Different lettered superscripts within each study indicates values were significantly different.

High-Density Lipoprotein Cholesterol Concentrations

The data related to the impact of hydrogenated fat/*trans* fatty acids compared to unhydrogenated oil /*cis* fatty acids on HDL cholesterol concentrations are less consistent than for LDL cholesterol concentrations (Tables 2, 3 and 4). As reported for LDL cholesterol concentrations, the effect of hydrogenated fat/*trans* fatty acids on HDL cholesterol concentrations, if present, is likely to be dose dependent (Judd et al., 1994). The preponderance of the data suggest that hydrogenated fat/*trans* fatty acids, relative to saturated fatty acids, result in lower HDL cholesterol concentrations (Ascherio et al., 1999; Zock and Mensink, 1996; Zock et al., 1995). Because of the potentially differential effects of hydrogenated fat/*trans* fatty acids on LDL and HDL cholesterol concentrations, concern has been raised regarding their effect on the total cholesterol or LDL cholesterol:HDL cholesterol ratio (Ascherio et al., 1999). However, with respect to dietary fat recommendations, the strategy to improve the total cholesterol or LDL cholesterol:HDL cholesterol ratio would not be different from that to decrease LDL cholesterol concentrations.

Lipoprotein(a) Concentrations

Lipoprotein(a) (Lp(a)) concentrations in plasma have been associated with increased risk for developing cardiovascular and cerebrovascular disease, possibly via inhibition of plasminogen activity (Lippi and Guidi, 1999; Nielsen, 1999; Wild et al., 1997). Lp(a) is a lipoprotein particle similar to LDL with respect to its cholesterol and apolipoprotein B100 content, but it also contains an additional apolipoprotein termed apo(a) (Lippi and Guidi, 1999; Nielsen, 1999). Lp(a) concentrations have been reported by some investigators to be increased after the consumption of diets enriched in hydrogenated fat/*trans* fatty acids (Tables 2, 3, and 4) (Almendingen et al., 1995; Aro et al., 1997; Lichtenstein et al., 1999; Mensink et al., 1992; Nestel et al., 1992b; Sundram et al., 1997), but not all (Chisholm et al., 1996; Judd et al., 1998; Lichtenstein et al., 1993; Louheranta et al., 1999; Müller et al., 1998). The magnitude of the mean increases in Lp(a) concentrations associated with *trans* fatty acid intake would not have a physiologically significant effect on cardiovascular disease risk. However, an unresolved issue at this time is the potential effect of relatively high levels of *trans* fatty acids in individuals with initially high concentrations of Lp(a).

Hemostatic Factors

The effect of *trans* fatty acids on hemostatic factors has been assessed by a number of investigators (Almendingen et al., 1996; Mutanen and Aro, 1997; Sanders et al., 2000; Turpeinen et al., 1998; Wood et al., 1993b) (see Table 5). In general, these researchers have concluded that hydrogenated fat/*trans* fatty acids had little effect on a variety of hemostatic variables. Similarly, Müller and colleagues (1998) reported that hemostatic variables were unaffected by the substitution of a vegetable oil-based margarine relatively high in saturated fatty acids when compared to a hydrogenated fish oil-based margarine.

Susceptibility of Low-Density Lipoprotein to Oxidation

Hydrogenated fat/trans fatty acids have consistently been reported to have little effect on the susceptibility of LDL to oxidation (Cuchel et al., 1996; Halvorsen et al., 1996; Nestel et al., 1992b; Sørensen et al., 1998) (Table 5).

TABLE 4 Dietary *Trans* Fatty Acids (TFA), Hydrogenated Fat, and Blood Lipid Concentrations: Free Living Trials

Reference	Study Population	Diet[a]	TFA (% of energy)	Blood Lipid Concentrations[c] LDL-C (mmol/L)	HDL-C (mmol/L)	Lp(a) (units/L)
Nestel et al., 1992a	26 mildly hypercholesterolemic men, 27–57 y	4-wk crossover, 42% fat				
		Control 1	3.8	4.13[c]	1.11[c]	
		Control 2	3.7	4.03[c,d]	1.15[c]	
		Blend 1	6.7	3.92[d,e]	1.10[c]	
		Blend 2	6.6	3.83[e]	1.11[c]	
Nestel et al., 1992b	27 mildly hypercholesterolemic men, 30–63 y	3-wk crossover, 36–37% fat				
		Control	< 1	4.22[c]	0.98[c]	235[c]
		18:1	1.4	3.90[d]	0.98[c]	236[c]
		TFA	5.7	4.27[c]	0.98[c]	296[d]
		16:0	< 1	4.16[c]	1.09[d]	249[e]
Seppänen-Laakso et al., 1993	57 men and women, middle-aged	12-wk crossover to 1 of 2 diets, 39–43% fat		Change from baseline	Change from baseline	
		Margarine	2.9	−0.20	+0.05	
		Rapeseed	0	−0.30	−0.01	
		Olive oil	0	−0.32	0.00	
Wood et al., 1993a	38 healthy men, 30–60 y	6-wk crossover, 38% fat				
		Butter	2.1	3.78[c]	1.22[c]	
		Butter-sunflower	1.0	3.49[d]	1.19[c]	
		Butter-olive	1.0	3.59[d]	1.22[c]	
		Hard margarine	11.1	3.47[d]	1.16[c]	
		Soft margarine	0	3.26[e]	1.16[c]	
Wood et al., 1993b	29 healthy men, 30–60 y	6-wk crossover, 37% fat				
		Butter	0.2	3.52[c]	1.03[c]	
		Crude palm	0	3.36[c]	1.03[c]	
		Margarine	3.0	3.36[c]	1.00[c]	
		Refined palm	0	3.41[c]	1.06[d]	
		Refined palm+sunflower	0	3.41[c]	1.03[c]	
		Sunflower oil	0	3.23[d]	1.00[c]	

TABLE 5 *Trans* Fatty Acid (TFA) Intake and Blood Clotting, Blood Pressure, and Low-Density Lipoprotein (LDL) Oxidation

Reference	Study Population	Diet[a]	TFA (% of energy)	Results[b]		Comments
Clotting						
Wood et al., 1993b	29 men, 30–60 y	6-wk crossover, 37% fat		TXB_2 (pg/mL)	6-keto-$PGF_{1\alpha}$ (pg/mL)	
		Butter	0.2	35[c]	89[d]	
		Crude palm oil	0	41[c]	94[c]	
		Margarine	3.0	40[c]	86[d]	
		Refined palm oil	0	40[c]	87[d]	
		Refined palm+sunflower	0	36[c]	100[c]	
		Sunflower oil	0	62[c]	95[c]	
Almendingen et al., 1996	31 men, avg 27 y	3-wk crossover, 33–36% fat		Fibrinogen (g/L)	PAI-1 activity (units/mL)	For PHSO, greater PAI-1 activity than PHFO or butter
		PHSO	8.5	3.0	13.5	Increased fibrinogen with butter diet
		PHFO	8.0	2.9	10.7	No significant difference in factor VII, fibrinogen peptide A, β-thromboglobulin, or tissue plasminogen activator
		Butter	0.9	3.1	8.8	
Mutanen and Aro, 1997	80 men and women, 20–52 y	5-wk crossover to 1 of 2 diets, 33–34% fat		Fibrinogen (g/L)		No marked difference in factor VII coagulation activity, tissue type plasminogen activity, or PAI-1 activity
		High 18:0	0.4	3.62		
		High TFA	8.7	3.61		
Turpeinen et al., 1998	80 men and women, 20–52 y	5-wk crossover to 1 of 2 diets, 32–34% fat				No difference in TXB_2 production or adenosine diphosphate-induced platelet aggregation in vitro
		18:0	0.4			Significant increase in collagen induced aggregation with 18:0 diet
		TFA	8.7			
Sanders et al., 2000	16 men and women, 18–32 y	1 test-meal crossover, 7% or 65% fat		$FVII_c$ (% standard)	$FVII_a$ (ng/mL)	No significant differences in factor VII coagulation activity; factor VII-activated concentrations were significantly higher with 18:1, 18:1 *trans*, 18:0, and 16:0 diets
		18:1	0.1	124	2.7	
		18:1 *trans*	24.7	122	1.9	
		18:0	0	114	1.9	

		16:0	0.2	112	2.1	
		MCT	0	112	1.5	
		Low fat	0	99	1.4	
Oxidation						
Cuchel et al., 1996	14 men and women, 44–78 y	32 d crossover, 30% fat				No difference in susceptibility to LDL oxidation
		Corn oil	0.44			
		Corn oil+margarine	4.16			
Halvorsen et al., 1996	29 men, 21–46 y	19-d crossover, 33–36% fat		Dienes (nmol/mg LDL)	Formation rate (nmol/mg LDL × min)	No significant differences in conjugated dienes, lipid peroxides, uptake by marcophages, or electrophoretic mobility of LDL
		Butter	0.9	1,020	10	TFA does not alter susceptibility to LDL oxidation
		PHSO	8.5	1,034	10	
		PHFO	8.0	1,107	10	
Sørensen et al., 1998	47 men, 29–60 y	4 wk, consumed 30 g/d of 1 of 2 margarines	(mol % of fat)	Dienes (nmol/g)	Oxidation rate (nmol/mg × min)	Fish oil consumption compared with sunflower oil margarine had no effect on LDL size and led to minor changes in LDL oxidation resistance
		Sunflower oil	0.79	445	10.4	
		Fish-oil, enriched	0.98	468	10.2	
Blood Pressure						
Mensink et al., 1991	59 men and women, 19–57 y, normo-tensive	3-wk crossover, 39–40% fat		SBP (mmHg)	DBP (mmHg)	No effect of TFA intake on blood pressure
		18:1	0	113	66	
		TFA	10.9	112	67	
		SFA	1.8	112	67	
Zock et al., 1993	55 men and women, 19–49 y	3-wk crossover, 40–43% fat		SBP (mmHg)	DBP (mmHg)	No effect of TFA intake on blood pressure
		18:2	0.1	114	68	
		18:0	0.3	113	70	
		TFA	7.7	113	69	

[a] PHSO = partially hydrogenated soy bean oil, PHFO = partially hydrogenated fish oil, MCT = medium-chain triacylglycerol, SFA = saturated fatty acid.
[b] TXB_2 = thromboxane B_2, 6-keto-$PGF_{1\alpha}$ = 6-keto-prostaglandin $F_{1\alpha}$, PAI-1 = plasminogen activator inhibitor type 1, $FVII_c$ = factor VII coagulant activity, $FVII_a$ = factor VII activated, SBP = systolic blood pressure, DBP = diastolic blood pressure.
[c,d] Different lettered superscripts within each study indicates values were significantly different.

Blood Pressure

A few reports addressed the issue of trans fatty acid intake and blood pressure (Mensink et al., 1991; Zock et al., 1993) (Table 5). The authors concluded that consumption of diets high in saturated, monounsaturated, or trans fatty acids resulted in similar diastolic and systolic blood pressures.

Coronary Heart Disease

Similar to saturated fatty acids, there is a positive linear trend between *trans* fatty acid intake and LDL cholesterol concentrations (Judd et al., 1994; Lichtenstein et al., 1999; Zock and Katan, 1992). Some evidence also suggests that *trans* fatty acids result in lower HDL cholesterol concentrations (Table 6). Hence, the net result is a higher total cholesterol (or LDL cholesterol):HDL cholesterol ratio (Judd et al., 1994; Lichtenstein et al., 1999; Zock and Katan, 1992). This finding, combined with data from prospective cohort studies (Ascherio et al., 1996; Gillman et al., 1997; Hu et al., 1997; Pietinen et al., 1997; Willett et al., 1993) (Table 6), has lead to the concern that dietary *trans* fatty acids are more deleterious with respect to coronary heart disease than saturated fatty acids (Ascherio et al., 1999).

Summary

There is a positive linear trend between *trans* fatty acid intake and total and LDL cholesterol concentration, and therefore increased risk of CHD, thus suggesting a Tolerable Upper Intake Level (UL) of zero. Because *trans* fatty acids are unavoidable in ordinary diets, achieving such a UL would require extraordinary changes in patterns of dietary intake. Such extraordinary adjustments may introduce other undesirable effects (e.g., elimination of foods, such as dairy products and meats, that contain *trans* fatty acids may result in inadequate intakes of protein and certain micronutrients) and unknown and unquantifiable health risks may be introduced by any extreme adjustments in dietary pattern. For these reasons, no UL is proposed. Nevertheless, it is recommended that *trans* fatty acid consumption be as low as possible while consuming a nutritionally adequate diet.

TABLE 6 Dietary *Trans* Fatty Acids (TFA): Epidemiological Studies

Reference	Study Design[a]	Dietary and Other Information	Results[b]			Comments
Lipoprotein concentration						
Siguel and Lerman, 1993	47 CAD cases 56 controls Case-control	No dietary intake information	Plasma TFA (%) HDL (mmol/L) LDL (mmol/L) TAG (mmol/L)	Case 1.38 0.88 3.78 1.78	Control 1.11 1.34 2.97 0.97	TFA negatively associated with HDL TFA positively associated with LDL and TAG
Coronary heart disease (CHD)						
Hudgins et al., 1991	76 men, 23–78 y Cross sectional	No dietary intake information	Total TFA in adipose tissue was 4.4% of total fatty acids			Total TFA content in adipose tissue was not significanlty related to risk factors of CHD (e.g, age, body mass index, LDL, cholesterol, blood pressure)
Troisi et al., 1992	748 men, 43–85 y Cross sectional	Food frequency questionnaire, multivariate analysis	TFA intake was directly related to total (r = 0.07, P = 0.04) and LDL (r = 0.09, P = 0.01) cholesterol			An increased TFA intake from 2.1 to 4.9 g/d increased the risk of MI by 27%
Willett et al., 1993	Women, 431 CHD cases Cohort, 8-y follow-up,	Food frequency questionnaire, multivariate analysis	TFA intake (% energy) 1.3 1.8 2.2 2.6 3.2	RR of CHD 1.0 1.4 1.25 1.55 1.8		Positive association with TFA intake and risk of CHD
Ascherio et al., 1994	239 MI cases 282 controls Case-control	Food frequency questionnaire, multivariate analysis	TFA intake (g/d) 1.69 2.48 3.35 4.52 6.51	RR of MI 1.0 0.73 1.24 1.63 2.28		Positive association of TFA intake and risk of myocradial infarction

continues

TABLE 6 Continued

Reference	Study Design[a]	Dietary and Other Information	Results[b]			Comments
Kromhout et al., 1995	12,763 men, 40–59 y Cohort, 25-y follow-up	Weighed food record	Correlation between 18:1*trans* intake and CHD mortality is 0.78 ($p < 0.001$)			
Ascherio et al., 1996	43,757 men, 40–75 y Cohort, 6-y follow-up	Food frequency questionnaire, multivariate analysis	TFA intake (g/d) 1.5 2.2 2.7 3.3 4.3	RR of MI 1.0 1.20 1.24 1.27 1.40		TFA intake directly associated with risk of MI
Hu et al., 1997	Women, 34–59 y 939 MI cases Cohort, 14-y follow-up	Food frequency questionnaire, multivariate analysis	TFA intake (% energy) 1.3 1.7 2.0 2.4 2.9	RR of MI 1.0 1.07 1.10 1.13 1.27		RR for 2% increment in energy from TFA intake was 1.93
Gillman et al., 1997	Men, 45–64 y 267 CHD cases Cohort, 21-y follow-up	24-h recall, multivariate analysis	Margarine intake (tsp/d) 0 1–4 ≥ 5	No. of events (/1,000) Period 1 77 42 18	Period 2 65 35 30	RR for CHD for each increment of 1 tsp/d was 0.99 for follow-up period 1 and 1.12 for period 2 Modest risk of CHD with increasing margarine intake
Pietinen et al., 1997	Smoking men, 50–69 y 1,399 coronary events 635 coronary deaths Cohort, 6.1-y follow-up	Food frequency questionnaire, multivariate analysis	TFA intake (g) 1.0 1.7 2.0 2.7 6.2	RR of major coronary event 1.00 1.10 0.97 1.07 1.14		Positive association between TFA intake and risk of coronary death

			TFA intake (g) 1.0 1.7 2.0 2.7 6.2	RR of coronary death 1.00 1.05 1.12 0.90 1.39	
Tavani et al., 1997	Women, 18–74 y 429 MI cases 866 controls Case-control	Questionnaire on selected indicator foods, multivariate analysis	Margarine intakes No or low Medium or high	RR of MI 1.0 1.5	The association with margaine could explain about 6% of MI in this population
Cancer					
Kohlmeier et al., 1997	Women, 50–74 y 291 breast cancer cases 407 controls Case-control	No diet information	Adipose TFA concentration TFA TFA within lowest PUFA tertile TFA within highest PUFA tertile	OR of breast cancer 1.46 3.65 0.97	Risk for breast cancer is based on the relative concentration of TFA and PUFA
Tuyns et al., 1988	35–75 y 453 colon cancer cases 365 rectal cancer cases 2,851 controls Case-control	Dietary history			There was no increased risk of either cancers from with increased consumption of margarine

[a] CAD = coronary artery disease, MI = myocardial infarction.
[b] HDL = high-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol, TAG = triacylglycerol, RR = relative risk, OR = odds ratio, PUFA = polyunsaturated fatty acid.

MAIRE JAARMA

STUDIES OF CHEMICAL AND ENZYMATICAL CHANGES IN POTATO TUBERS AND SOME HIGHER PLANTS CAUSED BY IONIZING RADIATION, INCLUDING STUDIES ON THE WHOLESOMENESS OF γ-IRRADIATED POTATO TUBERS AND EFFECTS ON SOME CARBOHYDRATES *IN VITRO*

STOCKHOLM 1967

STUDIES OF CHEMICAL AND ENZYMATICAL CHANGES IN POTATO TUBERS AND SOME HIGHER PLANTS CAUSED BY IONIZING RADIATION, INCLUDING STUDIES ON THE WHOLESOMENESS OF γ-IRRADIATED POTATO TUBERS AND EFFECTS ON SOME CARBOHYDRATES *IN VITRO**

MAIRE JAARMA

Biokemiska institutionen, Kungl. Universitetet i Stockholm, Stockholm, Sweden

This report is a survey of the publications listed below, which will be referred to by their Roman numerals I—XV. In addition some data from hitherto unpublished work are presented. The results concerning the influence of ionizing radiation on the different material studied will be discussed.

I. Jaarma, M.: Effects of chronic gamma irradiation on some sugars in *Vicia faba*. *Suomen Kemistilehti B* 28 (1955) 40—45;
II. Jaarma, M.: Influence of ionizing radiation on potato tubers. *Arkiv Kemi* 13 (1958) 97—105;
III. Jaarma, M.: The use of ^{110}Ag in quantitative paper chromatography of sugars. *Acta Chem. Scand.* 8 (1954) 860—862;
IV. Jaarma, M.: Influence of γ-irradiation on UDPglucose-fructose glucosyltransferase in potato tubers. *Acta Chem. Scand.* 20 (1966) 594—596;
V. Jaarma, M.: Influence of γ-irradiation on β-fructofuranosidase in potato tubers. *Acta Chem. Scand.* 20 (1966) 592—594;
VI. Hasselquist, H. and Jaarma, M.: Dünnschicht-chromatographische Bestimmung der Askorbinsäure in Kartoffelknollen. *Acta Chem. Scand.* 17 (1963) 529—533;
VII. Jaarma, M.: The influence of ionizing radiation on the proline content in potato tubers. *Acta Chem. Scand.* 20 (1966) 323—327;
VIII. Jaarma, M.: Studies on some chemical, enzymatical and physiological changes in plant material caused by γ-irradiation. *Arkiv Kemi*, in press;
IX. Jaarma, M.: Influence of ionizing radiation on oxidative phosphorylation, adenosinetriphosphatase and apyrase in potato tubers. *Acta Chem. Scand.* in press;
X. Jaarma, M.: Effects of ionizing radiations on inhibition of sprouting and biochemical and physiological changes in potato tubers. *Risö Report* No. 16 (1960) 70—72;
XI. Ehrenberg, L., Jaarma, M., and Zimmer, E. C.: The influence of water content on the action of ionizing radiation on starch. *Acta Chem. Scand.* 11 (1957) 950—956;
XII. Jaarma, M.: Determination of fatty acid composition in some pig fats by gas chromatography. *Acta Chem. Scand.* 18 (1964) 300—306;

and XIV) are: general health of the animals, growth rate, fertility, haematology, and activities of the enzymes ornithine carbamoyltransferase (E.C. 2.1.1.3) (OCT), aspartate aminotransferase (E.C. 2.6.1.1) (GOT), and alanine aminotransferase (E.C. 2.6.1.2) (GPT) in blood and liver of pigs. Histopathological investigations of organs were also performed. XV includes, moreover, studies on rats fed irradiated potatoes for three generations. The tendency towards faster growth rates of rats fed irradiated potatoes could be almost eliminated by adding sucrose to the control tubers in amounts corresponding to the increase caused by irradiation (XV, Fig. 2). Contrarily to the reaction in pigs (XIII, XIV), the tendency to an increased growth rate in rats fed irradiated potatoes was completely eliminated when steamed potatoes were fed (XV, Fig. 3). It should, however, be kept in mind, that also the changed amounts of amino acids (VII and VIII) could have been reflected in the growth rates of the animals. Thus, Burns *et al.* (63) have found a significantly increased growth rate in female rats fed irradiated potatoes which were steamed after irradiation.

No significant differences were observed in the activities of the enzymes investigated (OCT, GPT, GOT) between animals of the two groups. In other respects, too, the animals fed irradiated potatoes responded similarly to the animals fed control potatoes. Only two exceptions were found. The one was the different growth rates mentioned above, the other was a tendency towards faster increase of haemoglobin concentration in pigs fed irradiated potatoes (XIII, XIV). It should, however, be noted that the latter tendency is not common for all the pigs given the irradiated diet, but is found in certain individuals only. The reason for this varying reaction is still not clear. No corresponding haematological effect could be observed in rats (XIII, XV). In the blood of rats fed irradiated potatoes an increased level of some amino acids was, however, observed (VIII).

In spite of careful and long-term feeding investigations with animals, one could not be sure of the human reactions on irradiated potatoes. In the US Army, the Army Medical Service performed human feeding tests, which were reported in 1960 (64). 54 different foodstuffs were irradiated, most of them with doses of several megarads, including white potatoes irradiated at 10 and 20 kilorad, respectively. A total of 33 men, aged 19 to 24, participated in these tests. Each person consumed 9 or 10 irradiated foodstuffs for 15 days. During each study, and for a period of one year thereafter, each individual was carefully examined medically. No toxic effects whatsoever from irradiated foods were observed in the human test subjects, either during feeding or later.

Studies on haematological effects of γ-irradiated potatoes on human volunteers

In this investigation the normal blood values of seven haematologically healthy volunteers (4 males and 3 females) were determined in two succes-

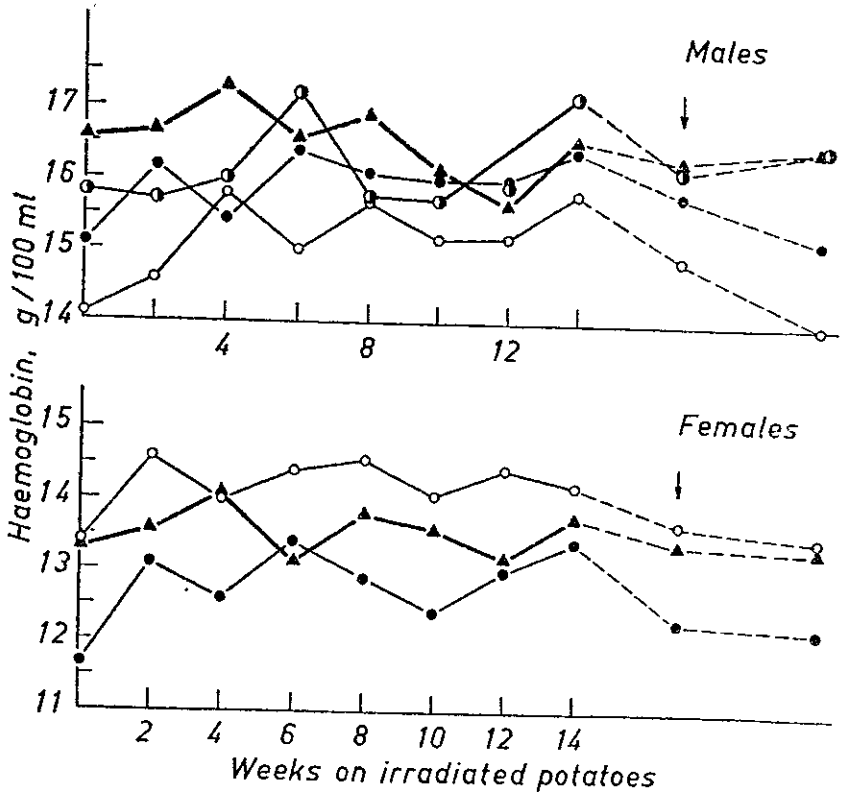


Fig. 2. Haemoglobin levels in blood of the human volunteers. Solid lines: during the period on irradiated potatoes; dashed lines: afterwards; arrow: mean of haemoglobin levels in two successive blood samples (one week interval).

sive blood samples taken by vein puncture, the one before onset of the potato diet, the second 10 days later. The percentage of haematocrit and haemoglobin as well as differential counts were determined. During the 10-day period non-irradiated potatoes (100—150 g/individual and day) were included in the normal diet of the volunteers. Thereafter the control tubers in the diet were replaced by the same amount of irradiated tubers (dose 14 kilorad), stored for at least two weeks after irradiation. Samples of blood were taken every second week and the determinations mentioned above were repeated. After 14 weeks, the irradiated potatoes were excluded from the diet and control tubers were given for further two weeks. In the next period blood was taken three times, first weekly and then one month later. From Fig. 2 it seems that all persons did not react in the same way, although no significance for interaction was found; see Table 1. An increased concentration of haemoglobin, especially in the first period of the investigation, was observed in all the individuals. The haematocrit concentrations are recorded in Table 2. The fluctuations were fairly parallel with those of haemoglobin. The differential counts were normal during the investigation period. Because of the varying haemoglobin con-

Table 1. Statistical analysis of haemoglobin values. a) period on control potatoes, b) period on irradiated potatoes, c) period after cessation of period b.

	Source of variance	Sum of squares	Degrees of freedom	Mean square
1)	Between periods a and b	5.77	1	5.77
	Between individuals	111.49	6	18.58
	Interaction	2.32	6	0.39
	Error	9.50	49	0.19
	Total	129.08	62	
2)	Between periods a and c	0.89	1	0.89
	Between individuals	91.43	6	15.24
	Interaction	0.80	6	0.13
	Error	3.42	28	0.12
	Total	96.54	41	
3)	Between periods b and c	1.89	1	1.89
	Between individuals	124.55	6	20.76
	Interaction	1.46	6	0.24
	Error	9.79	56	0.17
	Total	137.69	69	

1)	Quotients:	$\frac{5.77}{0.19} = 30.4$	$\frac{18.58}{0.19} = 97.79$	$\frac{0.39}{0.19} = 2.05$	$\frac{5.77}{0.39} = 14.79$
		$P < 0.001$	$P < 0.001$	$P > 0.05$	$P < 0.01$
		(1, 49)	(6, 49)	(6, 49)	(1, 6)
2)	Quotients:	$\frac{0.89}{0.12} = 7.42$	$\frac{15.24}{0.12} = 127.00$	$\frac{0.13}{0.12} = 1.08$	$\frac{0.89}{0.13} = 6.85$
		$P < 0.05$	$P < 0.001$	$P > 0.05$	$P < 0.05$
		(1, 28)	(6, 28)	(6, 28)	(1, 6)
3)	Quotients:	$\frac{1.89}{0.17} = 11.12$	$\frac{20.76}{0.17} = 122.12$	$\frac{0.24}{0.17} = 1.41$	$\frac{1.89}{0.24} = 7.88$
		$P < 0.005$	$P < 0.001$	$P > 0.05$	$P < 0.05$
		(1, 56)	(6, 56)	(6, 56)	(1, 6)

centrations at different dates (a quite normal variation) and the great individual variations, the results have been statistically analyzed. By variance analysis comparing the haemoglobin concentrations, 1:o *before* and *during* the "feeding" period, 2:o *during* and *after* the "feeding" period, it was evident that the haemoglobin values were significantly higher *during* the period than *before*. The values were also significantly higher *during* than

Table 2. Haematocrit concentrations in blood of the persons eating the potato diet.

Period	Haematocrit per cent						
	Females			Males			
before	41	37	40	42	44	49	45
during	42	39	41	44	48	48	47
	43	39	44	45	44	49	47
	42	38	40	44	48	49	44
	43	39	39	45	45	46	45
	40	38	40	43	44	46	45
	43	39	41	42	42	45	47
	40	39	40	42	45	48	48
	42	39	41	43	46	47	47
after	42	39	39	43	45	45	48
	40	37	40	42	45	46	48

after. An additional comparison of the values *before* with the values *after* shows that a small effect still remains; see Table 1. When all three periods were intercompared, P was found to be less than 0.001. The material has also been analyzed using the Scheffé contrast method. (Thanks are due to Mr. Göran Nilsson, Cand. Phil. and statistician by Statens Naturvetenskapliga Forskningsråd, who performed this analysis.) The same significances were obtained.

It should not be overlooked that the irradiated potatoes can have different effects on different individuals. It should also be kept in mind that other cooperative unknown factors could have contributed to the effect obtained. A study based on larger number of individuals is required before definite conclusions can be drawn. It should be pointed out that the statistical analyses were performed, though the material was small, because the possibilities of investigating human subjects haematologically after consuming irradiated potatoes are very rare. It would have been desirable, during the same experimental period, also to have had a corresponding number of males and females of the same ages consuming non-irradiated tubers. This control group should have been investigated parallel with the treated group in order to eliminate other factors which might have influenced the haemoglobin levels. Thus, the results should only be judged as tendencies in spite of the high significance obtained.

The doses necessary for irreversible inhibition of the sprouting of potatoes are low (6—10 kilorad) as compared with doses used for food preservation by irradiation (100 kilorad to several megarad). Nevertheless there are radiation induced chemical and enzymatical changes in the tubers also after low doses (II—IX; cf. *e.g.* 48, 50). In wholesomeness studies performed

Nutr. Dieta 8: 109–129 (1966)

From the Department of Animal Nutrition, Genetics, and Hygiene of The Royal Veterinary College (Head: Prof. *Sven Dyrendahl*), Stockholm; and the Biochemical Institute of The Royal University of Stockholm (Head: Prof. *Karl Myrbäck*)

On the Wholesomeness of γ-Irradiated Potatoes
II. Feeding Experiments with Pigs

Über die Zuträglichkeit von γ-bestrahlten Kartoffeln
II. Fütterungsversuche mit Schweinen

Sur l'intégrité de pommes de terre irradiées aux rayons γ
II. Expériences de nutrition sur des porcs

MAIRE JAARMA and GÖSTA BENGTSSON

Introduction

In a previous paper [6] it was stated that no obvious unfavourable effects could be observed in pigs and rats fed irradiated potatoes. All the facts noted in the investigation mentioned above indicated that the nutritional adequacy of the irradiated and control potatoes was equal. Some deviating effects were observed in the groups fed irradiated potatoes as compared to the groups fed control ones. The principal deviation concerned the haemoglobin levels, which tended to be higher in some of the animals fed irradiated potatoes.

The total number of pigs in the previous study seemed to be too small to provide reliable results. Thus we have found it necessary to repeat the investigation with larger groups of animals to ascertain whether the feeding of γ-irradiated potatoes to pigs could cause some toxic effects. The intention was also to clarify whether this diet, given for a longer period of time to pigs in two generations, has had any influence on the fertility or on the bacon quality of the animals. Furthermore, special care has been taken to establish some chemical, enzymatic, and morphological changes in the organisms of the experimental animals.

Several investigations on effects of feeding irradiated foods have been published during the past decade. In most of them, however, rats were used as test animals; only a few investigations have been performed with larger animals, such as dogs [14], and pigs [3, 11], or with human subjects [10].

For the sterilisation of foodstuffs by irradiation, doses of several megarads are required [17]. In feeding experiments with such products, irradiated at high dose levels, the search for toxic and undesirable effects is of quite another order than when potatoes are concerned, since only about 7–15 krad are required to achieve an inhibition of the sprouting of the tubers.

Larson et al. [8] found, when feeding irradiated vegetables (green beans and fruit compote) to dogs, a trend towards increased spleen weights in the animals receiving the irradiated (doses 3 and 6 Mrep) food. In addition to this, *Kalacheva* and *Sissakian* [7] have observed some influence of irradiation – with doses as low as 5–10 krad – on properties of mitochondria isolated from irradiated pea seedlings. Further, *Swaminathan et al.* [19] have found cytological abnormalities in barley embryos grown on potato mash irradiated with 20–80 krad of X rays.

Materials and Methods

Three common Swedish varieties of white potato, Bintje, King Edward, and Magnum Bonum, were selected for the investigation, and they were used in equal quantities. The main part of the tubers was irradiated in a ^{60}Co-γ-source at a dose rate of 150 r/s in a container taking 2 kg portions; a smaller part was irradiated at the lower dose rate, 620 r/h at a ^{137}Cs-γ-source. The dose in all experiments was 14–15 krad.

In the following the irradiated potatoes will be denoted I-potatoes, and the non-irradiated control potatoes C-potatoes. Accordingly the groups of animals fed irradiated potatoes will be denoted I-groups, and the groups fed non-irradiated ones C-groups. The potatoes given to both groups were cultivated under the same conditions and the irradiated ones were stored about one month before use. The potatoes were given steamed.

The animals used for the investigation were of Swedish Land Race; all of them were born and raised at the Department of Animal Nutrition, Genetics, and Hygiene of The Royal Veterinary College, Stockholm. Their state of health was controlled from birth throughout the whole experiment.

Fattening pigs. For this study 30 pigs were used. It was considered necessary to select piglets with the same initial weights for the investigation. As the total number of animals required was not available simultaneously, the feeding experiments were performed in two series: Series I comprising 21 piglets, and series II

9 piglets, respectively. Both series were divided into two groups. Division of the animals into I- and C-groups was based on sex and weight. The results of the previous investigation [6], however, have indicated that the initial haemoglobin levels of the animals should also be taken into consideration. Thus the animals were divided into the following groups, according to the three parameters: Weight, sex, and initial haemoglobin level:

Series I: I-group (6 ♀, 4 ♂); C-group (6 ♀, 5 ♂);
Series II: I-group (2 ♀, 3 ♂); C-group (2 ♀, 2 ♂).

The pigs in the I-group in series II were fed potatoes irradiated at the lower dose rate 620 r/h.

Each group of pigs was housed collectively and the diets were supplied on a communal basis. All these pigs were fed for a full period of fattening, from about 25 kg up to about 90 kg live weight. The composition of the ration is shown in Table I. Samples of blood were taken from the vena jugularis of all animals in series I once before introduction of the potato feeding, and then every second week, i.e. seven times during the investigation. The concentration of haemoglobin was determined by the cyanmethaemoglobin method with Drabkins reagent. All haemoglobin determinations were performed as duplicate analyses of the blood specimens. The method was tested for standard deviation by performing 20 parallel analyses of the same sample. The standard deviation for the determination of haemoglobin (g/100 ml blood, mean 15.20) was 0.09.

Table I

Fodder ration given to fattening pigs
Futterration der Mastschweine
Ration donnée aux porcs en cours d'engraissement

Week	Kg per pig and day	
	potatoes	feed concentrate[1]
1	0.5–1	1
2– 3	1 –1.3	1.25
4– 5	1.5–2.0	1.5
6– 8	2.5	2.0
9–10	3.0–3.5	2.0
11–13	4	2.0

[1] The feed concentrate consisted of 20% wheat bran, 4% meat meal, 4% fish meal, 1% fodder yeast, 1.5% chalk, 0.5% sodium chloride, 0.5% vitamins (A + D), 68.5% oats and barley (1:1).

The energy content of the mixture was 0.9 f.u. (Scandinavian feed units) or 2.66 megacalories convertible energy per kg. Scandinavian feed units have a nourishment value of approximately 1 kg normal barley for feeding.

The level of digestible raw protein was 121 g per kg feed concentrate. The amounts of calcium and phosphorus were 11.5 and 7.7 g per kg, respectively.

In the following the enzyme nomenclature recommended by the International Union of Biochemistry is used parallel with the older names of the enzymes or, in order to shorten the report, only the older names as abbreviations.

Systematic number and name	Recommended trivial name	Earlier name and abbreviation
E.C. 2.1.3.3. Carbamoylphosphate: L-ornithine carbamoyltransferase	Ornithine carbamoyltransferase	Ornithine carbamyltransferase; OCT
E.C. 2.6.1.1. L-aspartate: 2-oxoglutarate aminotransferase	Aspartate aminotransferase	Glutamic-oxaloacetic transaminase, glutamic-aspartic transaminase; GOT
E.C. 2.6.1.2. L-alanine: 2-oxoglutarate aminotransferase	Alanine aminotransferase	Glutamic-pyruvic transaminase, glutamic-alanine transaminase; GPT

Activity of OCT in the blood plasma (P) was estimated every second week by the microdiffusion technique described by *Reichard* [15] measuring the NH_3-formation at the arsenolysis of citrulline in borate buffer. In the first and the last weeks of the feeding period the activity of aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) was also determined in plasma (P) of the pigs according to *Reitman and Frankel* [16].

In the second series of fattening pigs the same determinations were performed as for the first one, and here blood cell counts were also made. Blood samples were taken at two occasions before introduction of the potato feeding and every second week during the feeding period.

Breeding pigs. Four female and two male piglets were given I-potatoes and another group of piglets with the same number and sex distribution was given C-potatoes. The pigs were raised to mating age on the following ration: 0.5–3.0 kg potatoes and 1.5–2.5 kg feed concentrate per pig and day. The highest amounts were given at the end of the period. The concentrate consisted of a mixture of 45% oats, 45% barley, and 10% Suggex®[1]. Its energy content was 1.0 f.u. (Scandinavian feed units) and the level of raw protein was 118 g per kg mixture.

[1] Suggex® is a special feed concentrate manufactured by AB Lactamin, Stockholm. Its composition is: Dried skim milk 10%, fish meal (70–74% raw protein) 40%, meat meal (min. 60% raw protein) 26%, wheat bran 10%, chalk 8%, sodium chloride and trace elements 5.5%, and vitamins 0.5%. The energy content is 0.86 f.u. and the raw protein level 450 g per kg concentrate.

Samples of blood were taken two times before introduction of potato feeding and three times during the feeding period. Determinations of haemoglobin concentrations, and of the activity of the enzymes mentioned above were performed. The mating period of the I- and C-groups commenced when the sows were about six and a half months old; the boars were just about one month younger and had been fed potatoes for a three-week shorter period. During this period and during pregnancy the I-sows were given 3.5 kg of irradiated potatoes and the C-sows the same amount of control potatoes per day. During the last month of pregnancy the feed concentrate ration was increased from 3.0 kg to 4.0 kg per sow and day. Its protein level was also increased to 140 g per kg by adding extra meat meal to the ration. During the last weeks of lactation the fodder ration was 4 kg of potatoes and 6 kg of feed concentrate in both I- and C-groups. Throughout the mating period and pregnancy no samples of blood were taken.

Table II

Results of mating for pigs fed I-potatoes and C-potatoes, respectively
Paarungsergebnisse der beiden Schweinegruppen
Résultats des accouplements entre les porcs nourris avec les pommes de terre I (irradiées) ou C

Parents ♂	♀	Offspring Number	Mean weight kg
I 152	I 103	9	1.76
I 150	I 108	11	1.75
I 150	I 117	11	1.38
I 150	I 119	12	1.40
Mean values		10.8	1.57
C 142	C 107[1]	(7)[1]	
C 142	C 115	11	1.31
C 146	C 118	12	1.49
C 146	C 123	9	1.55
Mean values		10.7	1.45

[1] This gilt slaughtered in early stage of pregnancy due to lameness in the hind legs. The number of foetuses not included in the mean value.

F_1-generation. The mating resulted in the offspring, shown in Table II. The health, appetite, vitality, and bodyweight for all these animals were followed from birth, and blood samples were taken from each animal in the 1st, 2nd, 3rd, and 10th week after birth. Haemoglobin concentration was determined. Two animals in each group were fed potatoes from 25 kg up to about 90 kg live weight. The fodder ration was the same as for the pigs in series I. The pigs from the I-sow were given irradiated

potatoes and the pigs from the C-sow were given control potatoes. Blood samples were taken and the haemoglobin level was examined at intervals of 2 to 3 weeks until slaughtering.

Post mortem examinations. In each feeding series, a number of animals was selected for *post mortem* examinations after slaughtering. Histopathological observations were made on samples from the stomach, duodenum, jejunum, colon, liver, kidneys, and heart. From the animals fed potatoes in two generations histological examinations of the spleen were performed, and liver and spleen weights were noted. All the carcases were classified according to routine methods (cf. p. 120). One of the carcases from each group was used for taste examination.

Results

One of the C-piglets in series I died in acute gastroenteritis four days after the introduction of the potato diet. In the breeding pig series, one pregnant C-sow was slaughtered owing to lameness in the hind legs. This sow had seven normally developed foetuses in the uterus. In other respects no difference in vitality or condition of health were observed between the animals in the I-group and the C-group, respectively, during the daily inspections. In the laboratory investigations performed, no deleterious effects on the animals fed irradiated potatoes could be shown. The detailed results are given below.

The growth rates for the pigs in the different experiments, including the F_1-generation, are shown in Figure 1, where the average gain of weights per week are given for both I- and C-groups. As mentioned in the previous paper [6], and as is also apparent in the present investigation, the growth of the animals in most of the I-groups was somewhat faster than in the corresponding C-groups. Statistical analysis was performed for series I of fattening pigs, since this comprises the largest number of animals. The irradiation did not cause any significant difference in the growth rate under the conditions of this investigation. The feed consumption per kg gain in this series was, however, 3.9 f.u. for the C-group and 3.5 for the I-group. This comparison was made for the time from the introduction of the potato diet until about 75 kg live weight.

The values for the blood cell counts in series II are shown in Table III. As can be seen from the mean values for the groups, no differences were observed. The mean values of the haemoglobin concentrations found for all the different groups of pigs are presented

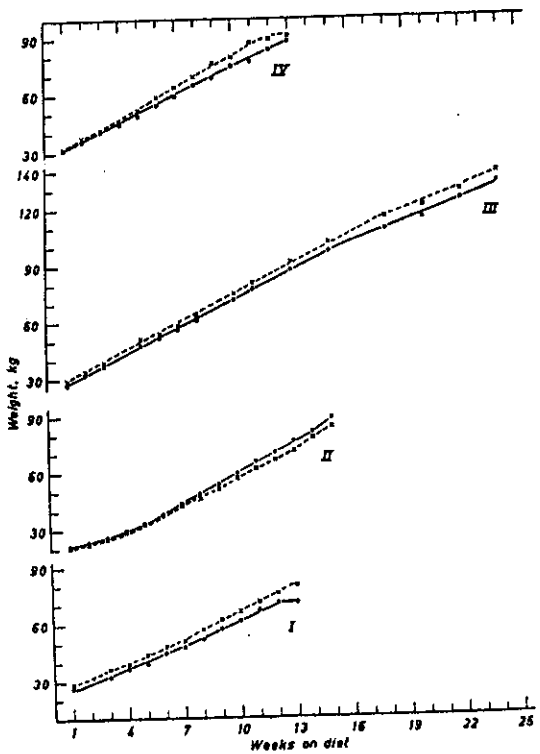


Fig. 1. The growth rate of pigs per week. I and II: Fattening pigs, series I and II; III: Breeding pigs (♀); IV: Pigs in the F_1-generation; Dashed lines: I-groups; Solid lines: C-groups. For series I $t = 0.97$, $d_f = 17$, $P > 0.05$ (cf. p. 114).

Abb.1. Wachstumsrate der Schweine pro Woche. I und II: Mastschweine der Reihen I und II; III: Zuchtschweine (♀); IV: Schweine der F_1-Generation; punktierte Kurve: I-Gruppe, ausgezogene Kurve: C-Gruppe.

Fig. 1. Courbe de croissance des porcs par semaine. I et II: porcs en cours d'engraissement, séries I et II; III: porcs (♀) gestantes; IV: porcs de la première génération F_1; courbes tiretées: groupes I; courbes continues: groupes C. Pour les séries I $t = 0{,}97$, $df = 17$, $P > 0{,}05$.

in Table IV. The corresponding values for the pigs in the second generation are shown in Table V. As can be seen from these tables, the mean values differ very little for pigs in I- and C-groups, respectively. There are, however, single animals in most of the I-groups, whose haemoglobin levels have increased at a faster rate and to a

Table III

Total number of red and white blood cells in series II
Mean values for the groups
Zahl der roten und weißen Blutzellen in Reihe II (Durchschnittswerte)
Nombre total de globules rouges et globules blancs, dans les séries II
(moyennes pour chaque groupe)

roup	Number of red blood cells in millions/mm³ weeks on potato diet									Number of white blood cells in thousands/mm³ weeks on potato diet							
	0	2	4	6	8	10	12	14	16	0	2	4	6	8	10	12	14
	6.8	6.7	6.7	6.4	6.5	6.8	7.2	7.1	6.7	18.0	26.9	26.7	24.5	26.5	22.2	22.0	20.0
	6.6	6.8	6.5	6.8	6.9	7.0	7.2	7.1	6.9	17.7	26.7	22.1	22.4	24.9	21.3	20.2	18.

Table IV

Haemoglobin values for pigs in the different groups
Hämoglobinwerte bei den verschiedenen Schweinegruppen
Taux d'hémoglobine pour les porcs des différentes séries

Groups	Haemoglobin g/100 ml; mean values for the groups weeks on potato diet										
	0	0	2	4	6	8	10	12	14	16	17
Fattening pigs I I	12.0	13.1	13.2	13.2	13.0	13.6	14.1				
Fattening pigs C I	12.2	13.0	13.2	12.9	12.7	13.3	13.8				
Fattening pigs I II	12.6	13.1	12.9	12.8	12.8	14.0	13.8	14.4	14.7	15.9	
Fattening pigs C II	12.6	13.2	13.0	12.8	13.3	14.5	14.2	14.9	14.9	15.9	
Breeding pigs I	10.9	10.4			11.8		13.5				14.0
Breeding pigs C	10.9	10.2			11.7		12.9				13.6

Table V

Haemoglobin values for pigs in the second generation
The parents were fed the potato diet, the sows until weaning of the piglets
Hämoglobinwerte der Schweine zweiter Generation
(Eltern mit Kartoffelfütterung, bei Sauen bis zum Abstillen der Ferkel)
Taux d'hémoglobine pour les porcs de seconde génération
Les parents sont nourris avec les pommes de terre, les truies jusqu'au sevrage des porcelets

mal up	Haemoglobin g/100 ml blood 43 I- and 32 C-pigs weeks after birth				2 I- and 2 C-pigs weeks on potato diet											
	1	2	3	10	2	4	5	7	8	10	11	12	13	14	15	
	8.4	9.6	11.1	11.1	11.5		12.0		12.5		13.0	12.9		12.8		
		9.4	11.1	12.0	12.0	11.8		13.2		12.9			13.1		13.0	

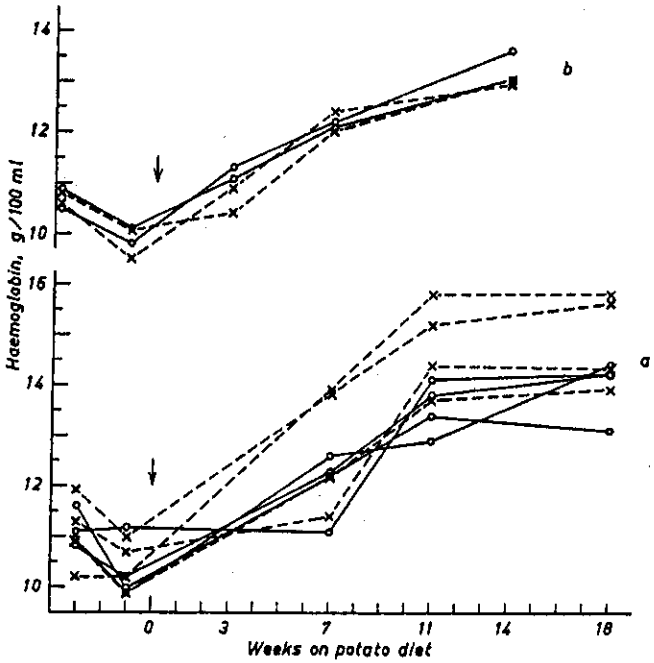


Fig. 2. Haemoglobin levels for the breeding pigs. a: Sows; b: Boars; Dashed lines: I-groups; Solid lines: C-groups; Arrow: Introduction of potato diet.
Abb. 2. Hämoglobinwerte der Zuchtschweine. a: Sauen; b: Eber. Punktierte Kurven: I-Gruppen; ausgezogene Kurven: C-Gruppen. Pfeile: Beginn der Kartoffelfütterung.
Fig. 2. Taux d'hémoglobine pour les truies gestantes. a: truies; b: porcelets. Courbes tiretées: groupes I; courbes continues: groupes C. La flèche indique le début du régime avec les pommes de terre.

higher value than the others. This fact is illustrated in Figure 2a, giving the haemoglobin values for the single sows in the breeding groups. This phenomenon was observed in one or several animals in all I-groups, with the exception of the boars in the breeding series, where the corresponding values were equal in the I- and C-groups (see Figure 2b).

The results of the enzymatic investigations are shown in Figure 3 and in Tables VI and VII. Figure 3 illustrates the mean values for the enzyme activities of ornithine carbamoyltransferase (OCT), alanine aminotransferase (GPT), and aspartate aminotransferase (GOT) in blood plasma (P) from the pigs in series II. The means and the ranges for PGPT- and PGOT-values are given, and the standard deviation

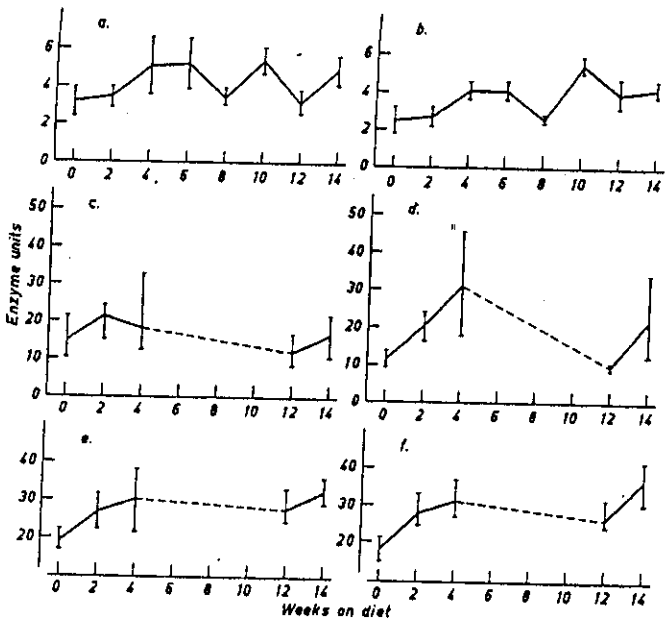


Fig. 3. Results of enzymatic determinations for fattening pigs in series II. a and b: POCT-values, mean ± standard deviation; c and d: PGOT-values, mean and range; e and f: PGPT-values, mean and range. I-group to the left, C-group to the right.

Abb. 3. Ergebnisse der Enzymbestimmungen bei Mastschweinen der Reihe II. a und b: POCT-Werte, Durchschnitt und Standardabweichungen. c und d: PGOT-Durchschnitt und Streuung. e und f: PGPT-Werte, Durchschnitt und Streuung. I-Gruppe links, C-Gruppe rechts.

Fig. 3. Résultats des déterminations enzymatiques pour les porcs en cours d'engraissement dans les séries II. a et b: valeurs de POCT: moyenne ± la déviation standard. c et d: valeurs de PGOT, moyenne et dispersion. Groupe I à gauche; groupe C à droite.

for POCT-activities at different dates of sampling is also noted. As can be seen from Figure 3, there is a good correlation in enzyme activity between I-group and C-group. The individual variations of the activity of POCT were found to be smaller in the C-group than in the I-group. The activities of PGOT and PGPT were determined at the beginning and at the end of the feeding period. The POCT-activities for the single animals in series I of the fattening pigs for the whole feeding period are shown in Table VI. The mean values for the

Table VI

Activities of ornithine carbamoyltransferase (OCT) in blood plasma from fattening pigs, series I

Aktivität der Ornithin-carbamoyltransferase (OCT) bei Mastschweinen. Reihe I

Activité de l'ornithine-carbamoyl-transférase (OCT) dans le plasma sanguin des porcs au régime. Séries I

Pig number and sex	Weeks on diet 0	2	4	6	8	10
	OCT-units of C-group					
3 ♀	8.8	8.2	9.4	7.8	9.4	12.1
7 ♀	5.6	11.0	11.6	6.1	6.1	5.5
11 ♀	2.2	5.0	4.4	2.2	4.9	—
18 ♀	5.0	4.4	5.5	6.6	5.0	Slaughtered
22 ♀	5.4	3.9	3.9	3.3	3.3	3.9
5 ♂	5.4	5.5	7.8	2.7	5.5	5.5
6 ♂	5.0	6.0	5.5	5.0	7.8	8.8
10 ♂	6.6	6.6	4.4	3.3	3.9	4.4
12 ♂	3.9	6.0	10.0	3.3	5.5	5.5
21 ♂	7.2	8.2	13.2	8.8	6.6	Slaughtered
Mean value	5.5	6.5	7.5	4.9	5.8	6.5
	OCT-units of I-group					
8 ♀	10.4	5.5	7.2	6.6	6.1	2.7
14 ♀	—	6.6	4.4	6.1	4.4	4.4
15 ♀	4.4	8.8	4.4	4.4	2.2	5.0
16 ♀	5.0	4.4	6.1	1.1	4.4	2.7
19 ♀	2.2	4.4	2.7	2.7	2.7	2.2
24 ♀	7.4	2.7	4.4	2.7	5.0	3.3
9 ♂	11.0	10.5	7.2	7.2	7.2	5.5
17 ♂	3.3	10.5	8.2	2.7	2.7	3.3
20 ♂	2.7	7.7	11.0	7.8	7.8	Slaughtered
23 ♂	4.4	5.0	5.5	3.9	3.9	3.9
Mean value	5.6	6.6	6.1	4.5	4.6	3.7

C-group are somewhat higher than for the I-group. The individual variation is, however, large. In Table VII the results of enzymatic determinations are recorded for the sows in the breeding series up to the period for mating. The PGOT-values for the animals in the I-group are slightly higher than in the C-group. The variations of the single values in both groups are large. There is a tendency towards

Table VII

Results of the enzymatic determinations for the breeding pigs (♀)
4 pigs in each group. Mean ± error of mean. Re abbreviations, see p. 112
Enzymwerte bei Zuchtschweinen (♀)
Résultats des déterminations enzymatiques des truies gestantes

Group	Enzyme	Weeks on diet 0	7	11	18
		Enzyme units			
I	POCT	3.3 ± 0.61	4.8 ± 1.06	6.7 ± 1.70	5.3 ± 1.17
C	POCT	4.8 ± 1.32	4.0 ± 0.23	7.1 ± 1.05	3.5 ± 0.18
I	PGOT	17.4 ± 1.95	25.2 ± 4.55	28.6 ± 4.70	24.4 ± 2.40
C	PGOT	17.9 ± 3.25	20.4 ± 1.30	25.2 ± 3.70	19.1 ± 3.70
I	PGPT	18.4 ± 0.90	23.4 ± 1.40	28.1 ± 2.70	38.8 ± 2.25
C	PGPT	19.6 ± 1.95	22.7 ± 1.25	26.6 ± 4.35	33.9 ± 4.95

an increase in the PGPT-values during the feeding period. The increase is valid for both of the groups.

Post mortem examinations. The results of the histopathological examinations of the pigs in series I are shown in Table VIII. No anomalous observations or differences between groups fed I-potatoes or C-potatoes were noted in this or any other series. Indications that the large quantities of potatoes fed to the animals *per se* might have caused pathological changes in the pigs were not observed. The liver and spleen weights for the pigs in the F_1-generation, given in per cent of body weight, were: Liver I-pigs 1.48, 1.57; C-pigs 1.71, 1.65; spleen I-pigs 0.21, 0.12; C-pigs 0.13, 0.11.

Classification of the bacon was carried out for all the animals in the different series. Class I (the thickness of the back fat 25–28 mm) was predominant; some of the animals were judged as *extra prima* (X) (< 25 mm), and some of them as class II (29–34 mm). Because of wide individual variations in the growth, especially at the end of the feeding period, the animals in series I were slaughtered on different dates. Owing to practical reasons, the exact values for the thickness of the back fat of these pigs were not obtained, whereas such statements were noted for the pigs in series II and in the F_1-generation.

Table VIII

Histopathological examination of organs from fattening pigs in series I
Histopathologische Befunde von Organen bei Mastschweinen. Reihe I
Examens histo-pathologiques des organes de porcs au régime dans les séries I

Animal number and sex	Liver	Kidney	Duodenum	Jejunum
9 ♂	0	Moderate, spotty, fatty infiltration of tubules	0	0
15 ♀	Slight, spotty proliferation of reticuloendothelial cells	0	Plenty of goblet cells; many cells of lamina propria with a great number of plasma cells	0
16 ♀	Moderate, fatty infiltration of Kupfer's cells	Slight, spotty, fatty infiltration of tubules	0	Slight, spotty fibro of lamina propria
17 ♂	Slight, fatty infiltration of Kupfer's cells in the peripheral parts of the lobules	As 9 ♂	0	0
23 ♂	0	0	0	0
24 ♀	0	0	0	0
5 ♂	0	As 16 ♀	0	0
7 ♀	0	As 9 ♂	Slight, spotty fibrosis of lamina propria	0
12 ♂	Moderate hyperemia	As 9 ♂	0	0
22 ♀	Slight, fatty infiltration of Kupfer's cells	As 9 ♂	As 15 ♀	0

N.B. No changes were found in myocardium, stomach, and colon.

The results of the classification are summarized in Table IX. The differences between the groups are not significant.

At taste examinations no divergences were noted between dishes prepared from pork, bacon, liver, kidneys, or hams, taken from the I-group pig or from the C-groupp ig.

Table IX

Results of the classification of carcases in series II and the F_1-generation
Ergebnisse der Schlachtfleisch-Klassifikation bei Reihe II und der F_1-Generation
Résultats de la classification des carcasses dans les séries II et dans la première génération

Group	Class[1]	Thickness of back fat mm	Mean value mm
I			
Series II	I	28	
	I	25	
	I	26	
	II	31	25.43
	I	27	
F_1-generation	X	18	
	X	23	
C			
Series II	I	29	
	I	26	
	II	31	28.33
	II	34	
F_1-generation	I	27	
	X	23	

$t = 1.29$; $d_f = 11$; $P > 0.05$.
[1] For explanation, see p. 120.

Discussion

As reported in the earlier paper by *Jaarma and Henricson* [6], no significant deleterious effects were noted in pigs fed irradiated potatoes. This result confirms the findings reported by other authors. Investigations on feeding γ-irradiated potatoes to pigs have been performed by *Horne and Hickman* [3]. Their study included 6 animals in each group. The dose rate is not mentioned. *Procházka and Černá* [11] also have fed γ-irradiated potatoes to pigs (42 animals), the dose used being 2500–240 000 rad; the dose-rate is not reported, but must have varied greatly as the potatoes were irradiated in batches of 500 kg. In none of the investigations were any deleterious effects noted in the animals fed the irradiated potatoes.

At the clinical observations in the present investigation no disturbances were observed in the health of the pigs in any of the experimental series. In one case the potato feeding was extended over a period of about 10 months (the sows in the breeding series) and the total amount of potatoes supplied amounted to about 900 kg per pig. The fattening pigs consumed 3.5 f.u. per kg weight gain in the I-groups and 3.9 in the C-groups. For conditions used in commerce in the intensive rearing of fattening pigs a feed consumption of about 3 f.u. per kg weight gain [18] is calculated. The somewhat high values found in this investigation can partly be explained by the fact that the pigs received an excess of energy with the large quantities of potatoes given. The potato ration was kept high with the intention of securing a maximum consumption.

No significant differences in the growth rates between I- and C-groups have been observed. There was a tendency towards faster growth in most of the I-groups. The only exceptions were the boars in the breeding series (two animals) and the fattening series II. In the last case, the tubers were irradiated at the lower dose rate 620 r/h. The influence of dose rates at irradiating biological subjects, including potatoes, is of vital importance. The necessity of taking the dose rates into consideration by irradiating biological objects has been thoroughly discussed by several authors [e.g. 1, 9, 12, 13].

The number of red and white blood cells was not influenced by feeding irradiated potatoes to the pigs. Nor were there any significant differences in the haemoglobin levels, the values for most of the I-groups, however, being somewhat higher than for the C-groups. The appreciably faster increasing haemoglobin levels and the higher final concentrations, which were noted for one or several animals in nearly all of the I-groups, is difficult to explain. The phenomenon is not necessarily a coincidence. The individual reaction to the I-feed, as reflected by increased haemoglobin values, could possibly be based on hereditary factors. Since the observation, however, is not characteristic for all the animals fed I-potatoes, the situation might arise from a number of cooperating factors, which contribute towards displacing the result in one or the other direction. In pigs the individual variations of the cellular metabolism are considerable and a component existing in minor amounts in the pig can perhaps be completed by an unknown factor in the irradiated potatoes.

In all toxicity studies, it must be borne in mind that irradiation induced changes in the feed can be extremely subtle. It was desirable, therefore, to include in the experimental plan a system of measure-

ments of the cellular metabolism. Such an approach is achieved through determinations of the activity of certain enzymes in the blood plasma of the animals fed the experimental diet. An assumed deleterious effect caused by the consumption of irradiated potatoes should be reflected primarily in changes in those organs which are responsible for the metabolism, the detoxication, and the secretion of possible injurious agents emanating from the potatoes. We, therefore, found it important to establish the possible existence of clinical or pathological changes in these organs.

The OCT-concentration in the liver is very high (*Wretlind et al.*, 22). A liver lesion is clinically reflected in a release of OCT and an increase of the OCT-activity in the blood plasma. In the same way a lesion of the heart, kidneys, or skeleton muscles, causes an increase of GOT-activity (aspartate aminotransferase) in the blood plasma, since these organs, as well as the liver, contain a high level of this enzyme (*Wretlind et al.*, 22). The determination of the GPT-activity (alanine aminotransferase) in the blood plasma gives, on the whole, the same result as the PGOT-test. In order to eliminate the possibility that injuries could have been caused to organs or tissues for which the GPT-levels are not known, the GPT-activity in the blood plasma was also determined for the majority of the pigs.

As a possible injurious effect may be expected preferentially in the liver, the POCT-activity was carefully investigated. No signs of liver lesions could be observed by the method used. Practically all single values for POCT-acticity in both I- and C-groups are within the ranges stated by *Wretlind et al.* [22] and *Tollertz* [21]. Enzyme activities higher than the mean value $\pm$ three times the standard deviation are considered to be pathological (*Tanhuanpää*, 20). The myocardium is not prejudicial affected, as reflected by the PGOT-values. Nor do the mean values for the PGPT-activities exceed the normal ranges, except the values for the sows in the breeding series, where the values for the I-group slightly exceed the upper limit. There is, however, also a slight increase in the PGPT-activities in the C-groups of the breeding pigs and in both groups of the fattening pigs. The increase appears in all cases at the end of the growth period. As this increase is found as well in the C-groups as in the I-groups, it can be associated with the fact that the pigs are entering into puberty at this time. It seems less probable that the potato ration *per se* should give rise to an increase of the PGPT-activity. No observations have been made in-

formating that fertility is affected by the I-potato diet. The numbers of matings per pregnancy have been equal for both groups and the pregnancy was reached at the normal age for gilts.

The determinations performed on the fattening pigs in the F_1-generation are in good agreement with the results from the other comparable series. During the five weeks (in average) of weaning no differences between the groups were noted. The I-group consisted of 43 piglets and the C-group of 32 piglets. After the lactation period the piglets were kept on conventional fodder for piglets and, again, no differences concerning general health, growth, or haemoglobin levels, were detectable. When four randomly selected F_1-pigs, two from each group, were fed I- and C-potatoes, respectively, until slaughter weight, the same tendency to faster increase of growth was observed in the I-group as earlier in series I and in the breeding series. The haemoglobin concentration for one of the pigs in the I-group was shown to increase strikingly faster and to a higher level than for the other three animals in the series. The higher spleen weight observed in the same I-group pig, as compared to that of the other three pigs in the F_1-generation, does not exceed the normal limits for spleen weights, but is mentioned as a parallel to the tendency of increased spleen weights in dogs noted by *Larson et al.* [8], as mentioned in the introduction.

There were no signs of morphological changes in parenchymatous organs nor in the digestive tract or spleen, as shown by the histopathological examinations. The slight to moderate fatty infiltration in the liver and the kidneys in some of the I-pigs and also in the C-pigs can be attributed to the high amount of carbohydrates in the diet. The ample presence of goblet cells in the duodenum of one pig in either group indicates an increased production of mucus in this part of the intestine, this finding, however, being of no importance. In the same two pigs there was observed a collection of cells in lamina propria of duodenum with strong interspersion of plasma cells. As numerous cells can normally be found unter the epithelium, the observations made on these two pigs need not be indicative of inflammatory changes.

In the grading of the carcases, no significant differences were noted between animals from I-groups and C-groups. The organoleptic examinations gave the same results for both groups. The bacon and organs from pigs fed I-potatoes had the same taste, appearance, and

structure as the corresponding parts from pigs fed C-potatoes. At the classifying a tendency towards a thinner back fat layer was noted for animals in the I-group as compared to the C-group. As the live weight at slaughter was nearly the same for all animals, this tendency may be interpreted as a better protein synthesis in the animals in the I-groups. The irradiation of the potato tubers causes a shift in the concentrations of many of the free amino acids present in the potato tubers [cf., e.g. 2, 5). A splitting of the proteins in the irradiated tubers make them more digestible. This in combination with the presence of an optimal set of free amino acids, might perhaps be the cause of an intensified protein synthesis in the animals.

Conclusions

The advantages of using ionizing irradiation for the prevention of sprouting in potatoes are well known. The prerequisite for permitting and recommending the irradiation of a foodstuff is, however, that a thorough investigation, including extensive feeding experiments, is performed on this foodstuff. This paper reports a part of such an investigation. As the results indicate, there is no evidence that irradiated potatoes are unwholesome or that they have any deleterious effects on pigs. Each species of animals has, however, a different metabolism and it must be assumed that the tolerance for foreign agents varies with the species. In order to ascertain the risks involved in feeding irradiated potatoes, the feeding experiments must be performed also with other species of animals, including man.

Summary

Three of the most common potato varieties in Sweden were irradiated at a sprout-preventing dose, 14–15 krad, at two different dose rates. Irradiated potatoes and non-irradiated control potatoes of the same variety were fed to fattening and breeding pigs (Swedish Land Race) in groups of equal numbers. The material used in the investigation comprised a total of 116 pigs in two generations. The potato feeding was introduced when the pigs had reached a weight of 25–30 kg, the ration of potatoes being gradually increased from 0.5 to 4 kg per pig and day. Each fattening pig received about 200 kg potatoes, each sow in the breeding series a total of 900 kg potatoes up to the end of the lactation, and each boar 600–700 kg.

The general health, the growth, and the fertility of the animals were observed. During the feeding period samples of blood were taken regularly. The tests included red and white blood cell counts, determination of the haemoglobin concentration, and of the activities of the enzymes ornithine-carbamoyltransferase (OCT), aspartate-aminotransferase (GOT), and alanine-aminotransferase (GPT) in the blood plasma. After slaughtering some of the organs were examined histologically. No divergence from the control animals could be observed. Quality tests of the slaughter products did not give any significant differences between the groups, nor could any other detrimental effects be found in the pigs fed the irradiated potatoes. A tendency towards faster growth in the pigs fed irradiated potatoes has been noted. In many of these animals, a faster increase in the haemoglobin level and a higher haemoglobin concentration have also been observed at the end of the experiment. Determinations of enzyme activities as well as blood cell counts gave the same results for both groups. The fertility and the number of pigs in each litter were also equal. Further, the second-generation pigs provided no indication that irradiated potatoes might give rise to deleterious effects.

ACKNOWLEDGEMENTS

The present investigation is a part of a research program, financially supported by *Fonden för främjande av forsknings- och försöksverksamheten på jordbrukets område*. Thanks are due to Dr. *N.-E. Björklund*, Department of Pathology of The Royal Veterinary College, Stockholm, for performing the histopathological examinations.

Zusammenfassung

Drei der gebräuchlichsten schwedischen Kartoffelsorten wurden mit keimtötenden Dosen, 14–15 krad, 2 verschiedenen Dosisleistungen bestrahlt. Bestrahlte und unbestrahlte (Kontrollen) Kartoffeln der gleichen Sorte wurden Mast- und Zuchtschweinen (schwedische Landrasse) in gleich großen Gruppen verfüttert. Es wurden 116 Schweine in 2 Generationen für die Untersuchung verwendet. Die Kartoffelfütterung begann bei Erreichen eines Gewichtes von 25–30 kg und stieg stufenweise von 0,5 auf 4 kg pro Schwein und Tag. Jedes Mastschwein erhielt etwa 200 kg Kartoffeln, jedes Mutterschwein der Zuchtserie bis zum Ende der Stillzeit etwa 900 kg und jeder Eber 600–700 kg.

Untersucht wurde allgemeiner Gesundheitszustand, Wachstum und Fertilität der Tiere. In regelmäßigen Abständen wurde Blut entnommen und untersucht: Zahl der roten und weißen Blutkörperchen, Hämoglobinkonzentration, Aktivitäten der Ornithin-Carbamoyltransferase (OCT), Aspartate-Aminotransferase (GOT) und Alanin-Aminotransferase (GPT). Nach dem Schlachten wurden einige Organe histologisch untersucht. Beide Gruppen ergaben gleiche Befunde. Qualitätsteste der Schlachtprodukte zeigten keine Unterschiede gegenüber den Kontrollen. Auch sonst konnten bei den mit bestrahlten Kartoffeln gefütterten Tieren keine Schäden festgestellt werden. Bei Tieren, die mit bestrahlten Kartoffeln gefüttert waren, konnte eine schnellere Wachstumstendenz beobachtet werden. Viele dieser Tiere zeigten rascheren Hämoglobinanstieg und größere Hämoglobinkonzentration bei Versuchsende. Beide Gruppen zeigten gleiche Enzymaktivitäten und Blutzellwerte. Ebenso waren Fertilität und Wurfgröße gleich. In der zweiten Generation traten keine Anhaltspunkte für eine schädigende Wirkung der bestrahlten Kartoffeln auf.

Résumé

Trois des variétés de pommes de terre les plus communes en Suède ont été irradiées à une dose empêchant le bourgeonnement, 14–15 krad, à deux différentes doses.

Les pommes de terre irradiées et les contrôles non irradiés de la même variété ont été donnés à des porcs en cours d'engraissement ou en gestation (Race Swedish Land) en nombre égal pour chaque groupe.

Le matériel comprenait pour cette expérience au total 116 porcs sur deux générations.

On a commencé à donner les pommes de terre au moment où les porcs ont atteint le poids de 25–30 kg, la ration de pommes de terre étant augmentée progressivement de 0,5 à 4 kg par porc et par jour. Chaque porc à l'engrais a reçu 200 kg de pommes de terre; chaque truie un total de 900 kg de pommes de terre jusqu'à la fin de la lactation et chaque porcelet 600 à 700 kg.

On a observé l'état de santé général, la croissance et la fertilité de ces animaux. Pendant la période d'alimentation contrôlée, on a prélevé régulièrement des échantillons de sang. Les examens comprenaient les numérations de globules rouges et blancs, la concentration d'hémoglobine et les déterminations des activités des enzymes ornithine-carbamoyltransférase (OCT) aspartate-aminotransférase (GOT), et alanine-aminotransférase (GPT) dans le plasma sanguin. Après l'abattage on a fait l'examen histologique de quelques organes. Nous n'avons constaté aucune différence avec les animaux témoins. Les tests de qualité des produits d'abattage n'ont pas donné de différences pour les porcs nourris avec les pommes de terre irradiées. On a noté une tendance à une croissance plus rapide des porcs nourris aux pommes de terre irradiées. Chez la plupart de ces animaux, en fin d'expérience, on a également observé une augmentation du taux d'hémoglobine et une plus forte concentration d'hémoglobine. Les déterminations d'activités enzymatiques et les numérations globulaires ont donné le même résultat pour les deux groupes. La fertilité et le nombre de porcs de chaque portée ont été identiques.

De plus, les porcs de seconde génération ne montraient aucun signe pouvant indiquer si les pommes de terre irradiées avaient pu donner lieu à des effets nocifs.

REFERENCES

1. *Heilinger, F.:* Über den Einfluß von Röntgenstrahlen auf Kleinwachstum und stoffliche Veränderungen in der Kartoffelknolle. Bayer. Landwirtsch. Jahrb. *42:* 69–77 (1965).
2. *Herrmann, J. und Raths, J.:* Stoffwechselveränderungen in Kartoffeln nach Behandlung mit ionisierenden Strahlen. Nahrung *2:* 1062–1090 (1958).
3. *Horne, T. and Hickman, J.R.:* Some notes on the wholesomeness of irradiated potatoes fed to pigs. Int. J. appl. Radiat. *6:* 255–257 (1959).
4. *Jaarma, M.:* Effects of ionizing radiations on inhibition of sprouting and biochemical and physiological changes in potato tubers. Risö Report *N:o 16:* 70–72 (1960).
5. *Jaarma, M.:* The influence of γ-irradiation on the content of free amino acids in potato tubers. To be published.
6. *Jaarma, M. and Henricson, B.:* On the wholesomeness of γ-irradiated potatoes. Acta vet. scand. *5:* 238–256 (1964).

7. *Kalacheva, V.Ya. and Sissakian, N.M.:* On the uncoupling of oxidation and phosphorylation in mitochondria of green plants after X-ray irradiation. Biokhimiya *30:* 858–863 (1964).
8. *Larson, P.S.; Belter, L.F.; Crawford, E.M.; Haag, H.B.; Finnegan, J.K. and Blackwell-Smith, R. Jr.:* Effects of adding gamma-irradiated green beans or fruit compote to the diet of dogs. Toxicol. appl. Pharmacol. *3:* 57–62 (1961).
9. *Natarajan, A.T. and Maric, M.M.:* The time-intensity factor in dry seed irradiation. Radiat. Botany *1:* 1–9 (1961).
10. *Plough, I.C.; Bierman, E.L.; Levy, L.M. and Witt, N.F.:* Human feeding studies with irradiated foods. Fed. Proc. *19:* 1052–1054 (1960).
11. *Procházka, Z. and Černá, J.:* The evaluation of the possibility to use by gamma rays retarded potatoes for the fattening of pigs. Vet. Med. *12:* 937–942 (1961).
12. *Rajewsky, B.:* Strahlendosis und Strahlenwirkung, pp. 21–24 (Georg Thieme, Stuttgart 1954).
13. *Rajewsky, B.; Aurand, K. and Wolf, I.:* Studies on time intensity factor after wholebody X-irradiation; in: *Hevesy, G.C. de; Forssberg, A.G. and Abbatt, J.D.:* Advances in Radiobiology, pp. 267–273 (Oliver & Boyd, London 1957).
14. *Reber, E.F.; Malhotra, O.P.; Kreier, J.P.; Norton, H.W. and Beamer, P.D.:* The effects of feeding irradiated flour to dogs. Toxicology *1:* 55–60 (1959).
15. *Reichard, H.:* Determination of ornithine carbamyl transferase with microdiffusion technique. Scand. J. clin. Lab. Invest. *9:* 311–312 (1957).
16. *Reitman, S. and Frankel, S.:* A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer. J. clin. Pathol. *28:* 56–63 (1957).
17. Summary Report of the FAO European Meeting of the Use of Ionizing Radiations for Food Preservation. Int. J. appl. Radiat. *6:* 8–13, especially p. 10 (1959).
18. Svinstamkontrollen. Kgl. Lantbruksstyrelsen, Med. Ser. B *N:o 23:* 22 (1955–1956).
19. *Swaminathan, M.S.; Chopra, V.L. and Bhaskaran, S.:* Cytological aberrations observed in barley embryos cultured in irradiated potato mash. Radiat. Res. *16:* 182–188 (1962).
20. *Tanhuanpää, E.:* Studies on the influence of dietary methyl linoleate on swine tissue lipids with special reference to nutritional muscular and liver dystrophy. Acta vet. scand. *6:* Suppl. 3 (1965).
21. *Tollertz, G.:* Studies on the tolerance to iron in piglets and mice. Dissertation, The Royal Veterinary College, Stockholm 1965.
22. *Wretlind, B.; Orstadius, K. and Lindberg, P.:* Transaminase and transferase activities in blood plasma and in tissues of normal pigs. Zentr. Veterinärmed. *6:* 963–970 (1959).

Author's address: Mrs. Maire Jaarma, Lic. Phil., Biokemiska institutionen, Kungl. Universitetet, Odengatan 63, *Stockholm Ö*s (Sweden)

Maternal Hemoglobin Concentration During Pregnancy and Risk of Stillbirth

Olof Stephansson, MD
Paul W. Dickman, PhD
Anna Johansson, MSc
Sven Cnattingius, MD, PhD

Context High and low maternal hemoglobin concentrations during pregnancy have been reported to increase risk of small-for-gestational-age (SGA) birth, which is a predictor of stillbirth. The relationship between hemoglobin concentration during pregnancy and risk of stillbirth is unclear.

Objective To study the associations among hemoglobin concentration at first measurement during antenatal care, change in hemoglobin concentration during pregnancy, and risk of stillbirth.

Design, Setting, and Participants Population-based, matched case-control study of births from 1987 through 1996 in Sweden including 702 primiparous women with stillbirths occurring at 28 weeks' gestation or later and 702 primiparous women with live births.

Main Outcome Measures Risk of stillbirth, classified as malformed or nonmalformed, antepartum or intrapartum, preterm or term, and SGA or non-SGA, compared by maternal hemoglobin concentration at first antenatal measurement and weekly changes in hemoglobin concentration during pregnancy, adjusted for maternal age, body mass index, height, smoking, socioeconomic status, and week of first hemoglobin measurement.

Results In multivariate analyses, compared with women with hemoglobin concentrations of 126 to 135 g/L at first antenatal measurement, women with concentrations of 146 g/L or higher were at increased risk of stillbirth (odds ratio [OR], 1.8; 95% confidence interval [CI], 1.0-3.3). This risk was slightly increased when the analysis was restricted to antepartum stillbirths without malformations (OR, 2.0; 95% CI, 1.1-3.8). When we further restricted the analyses to preterm and SGA antepartum nonmalformed stillbirths, the ORs increased to 2.7 (95% CI, 1.1-6.4) and 4.2 (95% CI, 1.3-13.9), respectively. Excluding women with preeclampsia and eclampsia further increased these risks. Average weekly change in hemoglobin concentration during early or late pregnancy was not significantly associated with risk of stillbirth, although a larger decrease in concentration tended to be protective. Anemia (hemoglobin concentration <110 g/L) was not significantly associated with risk of stillbirth in multivariate analyses (OR, 1.2; 95% CI, 0.5-2.7).

Conclusions High hemoglobin concentration at first measurement during antenatal care appears to be associated with increased risk of stillbirth, especially preterm and SGA antepartum stillbirths.

JAMA. 2000;284:2611-2617 www.jama.com

THE RELATIONSHIP BETWEEN MATERNAL hematological parameters and pregnancy outcome has been a source of continuing controversy. In developed countries, not only maternal anemia[1-3] but also high hemoglobin concentration during pregnancy[4-9] has been reported to increase the risks of unfavorable outcomes such as small-for-gestational-age (SGA) birth, preterm birth, and perinatal death. The associations between hemoglobin concentration in early pregnancy, changes in hemoglobin concentration during pregnancy, and risk of stillbirth are not known.[10]

In this large population-based case-control study, we investigated the associations between the hemoglobin concentration at the first measurement during antenatal care, weekly change in hemoglobin concentration during early and late pregnancy, and risk of stillbirth. Because the causes of stillbirth are varied, we also investigated hemoglobin-related risks in different subgroups of stillbirths.

METHODS

Description of Sample

From the population-based Swedish Medical Birth Register,[11] we obtained information on all single births to primiparous women living and giving birth within a geographically defined area in central Sweden from 1987 through 1996. Of 220 712 births, there were 725 stillbirths occurring at 28 weeks of gestation or later. For each case, we randomly selected 2 controls, matched by year and hospital of birth. Using the unique national registration number assigned to all Swedish residents, the standardized antenatal and obstetric records were retrieved from each of the 25 delivery archives and examined by one of us (O.S.), using a structured protocol. Of the 725 eligible cases, information on 702 (97%) was obtained: 10 cases were missing in the archives, 8 were incorrectly coded for delivery hospital, and 5 had an incorrect national registration number and were thereby

Author Affiliations: Department of Medical Epidemiology, Karolinska Institutet, Stockholm, Sweden. **Corresponding Author and Reprints:** Olof Stephansson, MD, Department of Medical Epidemiology, Karolinska Institutet, PO Box 281, SE-171 77, Stockholm, Sweden (e-mail: olof.stephansson@mep.ki.se).

impossible to trace. As for the control births, a second control birth was included only if the first selected control birth was not found. To obtain an equal number of case and control births (n=702), we included another 25 controls: 24 in the first control group were missing in the archives and 1 had an incorrect national registration number (retrieval rate, 96%). In 4 case and 2 control births, the mother had not received antenatal care. In 12 cases and 4 controls, the antenatal records were incomplete. The number of antenatal visits ranged from 0 to 34; mean and median number of visits were 12.2 and 12, respectively.

Information in antenatal records is prospectively recorded from the first to the last antenatal visit. At registration to antenatal care, we obtained information about maternal height, weight, occupation, and cigarette smoking status. During pregnancy, we repeatedly obtained information about blood pressure and proteinuria. From the obstetric and pediatric records, we recorded maternal age at delivery, gestational age at birth (or diagnosed stillbirth), weight at birth, and diagnosed malformations or chromosomal abnormalities. Gestational age was estimated using early second trimester ultrasound examination (generally before 19 gestational weeks) when available (94% of case and control births); otherwise, the last menstrual period was used.

Body mass index (BMI) at registration to antenatal care was calculated as weight in kilograms divided by the square of height in meters. A BMI of less than 20.0 was categorized as lean; 20.0 through 24.9 as normal weight; 25.0 through 29.9 as overweight; and 30.0 or more as obese. Information on mother's occupation was classified according to the Swedish Socioeconomic Classification[12] and grouped as blue-collar workers, low-level white-collar workers, intermediate and high-level white-collar workers, students, and others (54 were not in the labor force, 12 were self-employed, and 80 were not classifiable). Maternal smoking status was categorized as no daily smoking, 1 to 9 cigarettes per day, or 10 or more cigarettes per day.

Preeclampsia and eclampsia were defined according to the criteria given by the National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy.[13] Mild preeclampsia was defined as gestational hypertension (blood pressure ≥140/90 mm Hg in ≥2 readings ≥6 hours apart, occurring after 20 weeks of gestation) accompanied by mild or moderate proteinuria (≥2 urinary dipsticks with 1+ or 2+ or 300 mg to 3 g of protein in a 24-hour urine collection). Severe preeclampsia was defined as gestational hypertension accompanied by severe proteinuria (≥2 urinary dipsticks with 3+ or ≥3 g of protein in a 24-hour urine collection), or gestational hypertension with a diastolic blood pressure of at least 110 mm Hg (in ≥2 readings ≥6 hours apart), regardless of proteinuria. Eclampsia was defined as seizures in a patient with preeclampsia that could not be attributed to other causes.

Blood was taken for hemoglobin concentration estimation repeatedly during pregnancy. The mean gestational week of the first hemoglobin concentration measurement was 10.5 for cases and controls. There are no uniform criteria for categorization of hemoglobin measures during pregnancy. Based on a previous report, we divided hemoglobin concentration taken during antenatal care into 5 categories.[9] A separate stratification was performed to address the question of anemia (<110 g/L) and risk of stillbirth.[14] The average weekly change in hemoglobin concentration during pregnancy was estimated separately for early and late pregnancy periods using simple linear regression. The start of the early period was identified as the week that the first hemoglobin concentration measurement was taken, and the end of the early period was identified as the week of gestation closest to week 25, provided it was between week 17 and week 28. The regression line for the early period was fitted to all hemoglobin measurements during the period (the mean number of hemoglobin measurements was 2.9). The estimated weekly change in concentration was given by the slope of the fitted regression line. The method of estimating average change in hemoglobin concentration for the late period was similar; the start of the late period was identical to the end week of the early period, and the end week was defined as the week of the last concentration measurement in pregnancy (the mean number of hemoglobin measurements was 3.1). Average weekly change in hemoglobin concentration was not estimated if the difference between the start and end dates of the respective period was less than 3 weeks. Among cases, we only considered hemoglobin concentration measurements prior to stillbirth or prior to the birth week of the matched control. Among controls no hemoglobin concentration measurements were used after gestational week of stillbirth for the matched case. Average weekly change in hemoglobin concentration in early and late pregnancy was divided into 6 categories.

Stillbirths were classified as malformed or nonmalformed, antepartum or intrapartum, preterm or term, and SGA or non-SGA. Malformations were only noted if they were lethal or potentially lethal.[15] Preterm birth was defined as gestational age less than 37 completed weeks, and SGA was defined as birth weight more than 2 SDs below the mean birth weight for gestational age, according to the Swedish birth weight curve in common use.[16]

Statistical Methods

We used standard statistical methods for the analysis of matched case-control studies, namely conditional logistic regression models estimated using SAS PROC PHREG.[17] The data were individually matched, but some matched pairs had identical values for the matching variables. We therefore analyzed the data as if they were N:M matched, ie, each stratum contained N controls and M cases where neither N nor M were necessarily 1. All 702 controls were eligible for the analysis but

were only included if they belonged to a stratum that contained at least 1 case. For each model, observations with missing values for explanatory variables were excluded from the analysis, although the corresponding matched case or control was included in the analysis if the stratum contained at least 1 case and 1 control with full covariate information. The number of observations excluded due to missing values was small (TABLE 1). Odds ratios (ORs) with 95% confidence intervals (CIs) were used to estimate the relative risk.

The study was approved by the research ethics committee at Karolinska Institutet, Stockholm, Sweden. The committee did not require informed consent because the information had been collected in hospital archives; however, no personal identification numbers were used in the databases.

RESULTS

Low- (≤115 g/L) and high- ≥146 g/L) hemoglobin concentrations at first antenatal measurement were associated with increased risk of stillbirth in the univariate analyses (Table 1). Twenty cases and 15 controls were anemic (<110 g/L), and the OR for stillbirth related to anemia was 1.5 (95% CI, 0.7-3.0). Compared with women with a moderate decrease in hemoglobin concentration during early or late pregnancy, women with the largest increase in hemoglobin concentration were at increased risk of stillbirth. The risk of stillbirth increased with advancing maternal age, increasing BMI, low maternal stature, smoking, low socioeconomic status, and severe preeclampsia or eclampsia.

The relationship between hemoglobin concentration at first antenatal measurement and average change in hemoglobin concentration during pregnancy is shown in the FIGURE. In early pregnancy, mean hemoglobin concentrations decreased, with the exception of the group in the lowest concentration (≤115 g/L) category. Some of the results demonstrated in the Figure can be explained by regression to the mean, that is, the tendency of observations that are extreme by chance to move closer to the mean when repeated. However, those women who began pregnancy with a high hemoglobin concentration continued to have higher concen-

Table 1. Characteristics of Primiparous Women With Stillbirth (Cases) and Matched Controls, Sweden 1987-1996*

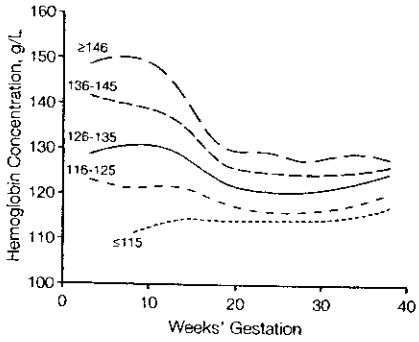
Characteristics	No. (%) Cases (n = 702)	No. (%) Controls (n = 702)	OR (95% CI)
First hemoglobin concentration, g/L			
≤115	73 (10.4)	47 (6.7)	1.8 (1.2-2.6)
116 to 125	176 (25.1)	189 (26.9)	1.1 (0.8-1.4)
126 to 135†	231 (32.9)	263 (37.5)	1.0
136 to 145	165 (23.5)	167 (23.8)	1.1 (0.8-1.5)
≥146	39 (5.6)	25 (3.6)	1.8 (1.1-3.2)
Data missing	18 (2.6)	11 (1.6)	. . .
Early pregnancy weekly change in hemoglobin concentration, g/L			
≤−1.6	73 (10.4)	70 (10.0)	1.1 (0.7-1.7)
−1.5 to −1.1†	107 (15.2)	114 (16.2)	1.0
−1.0 to −0.6	186 (26.5)	197 (28.1)	1.0 (0.7-1.4)
−0.5 to −0.1	128 (18.2)	158 (22.5)	0.9 (0.6-1.2)
0.0 to 0.4	89 (12.7)	84 (12.0)	1.1 (0.8-1.7)
≥0.5	51 (7.3)	26 (3.7)	2.2 (1.3-3.7)
Data missing	68 (9.7)	53 (7.5)	. . .
Late pregnancy weekly change in hemoglobin concentration, g/L			
≤−0.6	103 (14.7)	98 (14.0)	1.4 (0.9-2.2)
−0.5 to −0.1†	68 (9.7)	87 (12.4)	1.0
0.0 to 0.4	106 (15.1)	100 (14.2)	1.3 (0.9-2.0)
0.5 to 0.9	64 (9.1)	83 (11.8)	1.0 (0.6-1.6)
1.0 to 1.4	52 (7.4)	45 (6.4)	1.6 (0.9-2.6)
≥1.5	68 (9.7)	51 (7.3)	1.8 (1.1-2.9)
Data missing	241 (34.3)	238 (33.9)	. . .
Age, y			
≤19	33 (4.7)	45 (6.4)	0.9 (0.5-1.4)
20 to 24†	203 (28.9)	244 (34.8)	1.0
25 to 29	261 (37.2)	253 (36.0)	1.2 (1.0-1.6)
30 to 34	138 (19.7)	123 (17.5)	1.4 (1.0-1.9)
≥35	67 (9.5)	37 (5.3)	2.2 (1.4-3.5)
Body mass index, kg/m²			
≤19.9†	74 (10.5)	105 (15.0)	1.0
20.0 to 24.9	393 (56.0)	442 (63.0)	1.3 (0.9-1.8)
25.0 to 29.9	147 (20.9)	103 (14.7)	2.0 (1.3-3.0)
≥30.0	57 (8.1)	31 (4.4)	2.5 (1.5-4.3)
Data missing	31 (4.4)	21 (3.0)	. . .
Height, cm			
≤159	104 (14.8)	71 (10.1)	1.5 (1.0-2.1)
160 to 164	179 (25.5)	182 (25.9)	1.0 (0.7-1.3)
165 to 169†	208 (29.6)	203 (28.9)	1.0
≥170	186 (26.5)	228 (32.5)	0.8 (0.6-1.1)
Data missing	25 (3.6)	18 (2.6)	. . .
Cigarette smoking at registration to antenatal care			
Nonsmoker†	483 (68.8)	541 (77.1)	1.0
1-9 cigarettes/d	114 (16.2)	90 (12.8)	1.4 (1.1-1.9)
≥10 cigarettes/d	87 (12.4)	58 (8.3)	1.7 (1.2-2.4)
Data missing	18 (2.6)	13 (1.9)	. . .

Table 1. Characteristics of Primiparous Women With Stillbirth (Cases) and Matched Controls, Sweden 1987-1996* (cont)

Characteristics	No. (%) Cases (n = 702)	Controls (n = 702)	OR (95% CI)
Occupation			
Blue-collar worker	297 (42.3)	257 (36.6)	1.5 (1.2-2.0)
Low-level white-collar worker	124 (17.7)	113 (16.1)	1.5 (1.0-2.0)
Intermediate–high-level white-collar worker†	151 (21.5)	200 (28.5)	1.0
Student	61 (8.7)	55 (7.8)	1.5 (1.0-2.3)
Other	69 (9.8)	77 (11.0)	1.2 (0.8-1.8)
Preeclampsia or eclampsia			
No†	614 (87.5)	639 (91.0)	1.0
Mild preeclampsia	48 (6.8)	40 (5.7)	1.2 (0.8-1.9)
Severe preeclampsia or eclampsia	40 (5.7)	23 (3.3)	1.8 (1.1-3.0)

*Percentages may not add to 100 due to rounding. Ellipses indicate not applicable; OR, odds ratio; and CI, confidence interval.
†Reference group.

Figure. Hemoglobin Concentration During Pregnancy



Data are grouped by hemoglobin concentration at first measurement during antenatal care. Primiparous women with stillbirth and matched controls, Sweden 1987-1996. Cubic splines were fitted to data grouped into 5-week intervals.

trations throughout pregnancy and vice versa. The lowest hemoglobin concentrations were found close to the 30th week of gestation for all groups. In late pregnancy, there was a small increase in mean hemoglobin concentrations, with the exception of the group with the highest hemoglobin concentration at first antenatal measurement (≥146 g/L). We also wanted to investigate whether cases and controls within different first hemoglobin categories differed in the change of hemoglobin values during pregnancy. We therefore repeated the analysis in the Figure separately for cases and controls, but the changes in hemoglobin values from first hemoglobin concentration were essentially the same (data available on request).

Women with a high first hemoglobin measurement were more likely to have a high BMI, preeclampsia or eclampsia, whereas there was no clear association between first-hemoglobin measurement and maternal age, cigarette smoking, or occupational status (data available on request).

Next, we performed conditional logistic regression analysis on the associations between first-hemoglobin concentration, early pregnancy change in hemoglobin values, and risk of stillbirth (TABLE 2). Compared with women with mid-range first hemoglobin concentrations (126-135 g/L), women with the highest hemoglobin concentrations were at increased risk of stillbirth (OR, 1.8). Anemia (<110 g/L) (16 cases and 13 controls) was not significantly associated with risk of stillbirth (OR, 1.2; 95% CI, 0.5-2.7). The subgroups of malformed and intrapartum stillbirths were too small to be analyzed separately, and we assumed that these groups were not primarily associated with hemoglobin concentration during pregnancy. We therefore restricted the analysis to antepartum stillbirths without malformations. This restriction slightly increased the risk of stillbirth related to high first hemoglobin concentration (OR, 2.0). There was no significant interaction between first hemoglobin concentration and any of the covariates in the model. Women with the largest increase in hemoglobin concentration in early pregnancy were at increased risk of stillbirth, but change in hemoglobin concentration in early pregnancy was not statistically significant in any model. Hemoglobin concentration at first measurement during antenatal care and change in hemoglobin concentration in early pregnancy were also analyzed separately (after adjusting for the confounders included in Table 2) with no major differences in results.

Antepartum stillbirths without malformations were also analyzed according to birth weight for gestational age (TABLE 3). For SGA stillbirths, a first hemoglobin measurement of 146 g/L or higher was associated with a more than 4-fold increase in risk, and when women with preeclampsia or eclampsia were excluded, this risk was substantially increased (OR, 15.1). We found no significant association between first hemoglobin level and non-SGA stillbirths ($P=.19$). Change in hemoglobin concentration during early pregnancy was not significantly associated with risks of SGA or non-SGA stillbirths ($P=.18$ and $P=.13$, respectively).

We also stratified nonmalformed antepartum stillbirths by gestational age (TABLE 4). For preterm stillbirth, women with a first hemoglobin level of 146 g/L or higher had an almost 3-fold increase in risk, and this risk was further increased when women with preeclampsia or eclampsia were excluded. There was no significant association between term stillbirth and first hemoglobin concentration ($P=.14$), or change in hemoglobin during early pregnancy and risk of preterm or term stillbirth ($P=.17$ and $P=.08$, respectively).

Finally, we introduced change in hemoglobin concentration during late pregnancy in 3 models of stillbirth (all, nonmalformed, and antepartum nonmalformed stillbirth). Change in hemoglobin concentration during early and late pregnancy was not statistically significant in any model, indicat-

Table 2. First Hemoglobin Concentration and Early Pregnancy Weekly Change in Hemoglobin Concentration for Various Categories of Stillbirth*

Characteristics	All Stillbirths (n = 615/627) OR (95% CI)	*P* Value†	Stillbirths Without Malformations (n = 555/618) OR (95% CI)	*P* Value†	Antepartum Stillbirths Without Malformations (n = 519/610) OR (95% CI)	*P* Value†
First hemoglobin concentration, g/L						
≤115	1.6 (1.0-2.6)	.09	1.7 (1.0-2.8)	.05	1.7 (1.0-2.8)	.03
116 to 125	1.0 (0.7-1.3)		1.0 (0.7-1.3)		0.9 (0.6-1.2)	
126 to 135‡	1.0		1.0		1.0	
136 to 145	1.0 (0.7-1.4)		1.1 (0.8-1.5)		1.0 (0.7-1.4)	
≥146	1.8 (1.0-3.3)		2.0 (1.1-3.7)		2.0 (1.1-3.8)	
Early pregnancy weekly change in hemoglobin concentration, g/L						
≤−1.6	1.1 (0.7-1.7)	.14	1.0 (0.7-1.7)	.14	1.1 (0.6-1.7)	.09
−1.5 to −1.1‡	1.0		1.0		1.0	
−1.0 to −0.6	1.0 (0.7-1.5)		1.1 (0.7-1.5)		1.1 (0.8-1.6)	
−0.5 to −0.1	0.9 (0.6-1.3)		0.8 (0.6-1.2)		0.8 (0.6-1.2)	
0.0 to 0.4	1.3 (0.8-2.0)		1.2 (0.7-1.9)		1.2 (0.8-2.0)	
≥0.5	1.9 (1.0-3.4)		1.9 (1.0-3.5)		2.1 (1.1-4.0)	

*Adjusted for maternal age, height, occupation, smoking, body mass index, and week of first hemoglobin concentration measurement. First hemoglobin concentration is adjusted for early pregnancy weekly change in hemoglobin concentration and vice versa; n indicates number of cases/controls; OR, odds ratio; and CI, confidence interval.
†Wald test of the overall effect (test of general heterogeneity).
‡Reference group.

ing that first hemoglobin concentration was a stronger predictor for stillbirth compared with changes in hemoglobin concentration during early or late pregnancy.

COMMENT

The results from this study suggest that a high hemoglobin concentration in early pregnancy is associated with an almost 2-fold increase in risk of stillbirth. The risk increase is even larger among specific stillbirth subgroups, such as antepartum nonmalformed preterm or SGA stillbirths. When cases and controls with preeclampsia or eclampsia were excluded, these risks were further increased. Change in hemoglobin concentration during early or late pregnancy did not significantly influence stillbirth risk. Because anemia (<110 g/L) in early pregnancy is rare in Sweden,[18] our study did not have sufficient power to investigate the association between anemia and stillbirth.

This population-based case-control study included more than 700 cases of stillbirth and 700 controls, for which 96% to 97% of the medical records were retrieved. Because exposure was registered prospectively during pregnancy, recall bias is unlikely. Possible confounders such as maternal age, BMI, smoking status and socioeconomic status were accounted for in the analyses. The relatively homogeneous population in Sweden, the standardized antenatal care, and the use of uniform records further minimized the possibility of confounding due to differences in sociodemographic factors or pregnancy management. We investi-

Table 3. Small-for-Gestational-Age (SGA) Antepartum Stillbirths Without Malformations Before and After Excluding Women With Preeclampsia or Eclampsia*

Characteristic	SGA Antepartum Stillbirths Without Malformations: All Births (n = 137/390) OR (95% CI)	*P* Value†	Preeclampsia and Eclampsia Excluded (n = 121/316) OR (95% CI)	*P* Value†
First-hemoglobin concentration, g/L				
≤115	1.5 (0.6-3.9)	.003	2.7 (0.9-7.7)	<.001
116 to 125	0.4 (0.2-0.8)		0.5 (0.2-1.2)	
126 to 135‡	1.0		1.0	
136 to 145	1.1 (0.6-2.1)		1.7 (0.8-3.6)	
≥146	4.2 (1.3-13.9)		15.1 (3.0-75.5)	
Early pregnancy weekly change in hemoglobin concentration, g/L				
≤−1.6	0.9 (0.3-2.5)	.18	0.8 (0.3-2.7)	.41
−1.5 to −1.1‡	1.0		1.0	
−1.0 to −0.6	1.7 (0.8-3.5)		1.7 (0.7-4.1)	
−0.5 to −0.1	1.2 (0.6-2.6)		1.3 (0.5-3.2)	
0.0 to 0.4	2.6 (1.1-6.4)		2.8 (0.9-8.2)	
≥0.5	3.3 (1.0-11.5)		2.2 (0.6-9.1)	

*Adjusted for maternal age, height, occupation, smoking, body mass index, and week of first hemoglobin concentration measurement. First hemoglobin concentration is adjusted for early pregnancy weekly change in hemoglobin concentration and vice versa; n indicates number of cases/controls; OR, odds ratio; and CI, confidence interval.
†Wald test of the overall effect (test of general heterogeneity).
‡Reference group.

Table 4. Preterm Antepartum Stillbirths Without Malformations Before and After Excluding Women With Preeclampsia or Eclampsia*

	Preterm Antepartum Stillbirths Without Malformations			
	All Births (n = 248/494)		Preeclampsia and Eclampsia Excluded (n = 216/422)	
Characteristics	OR (95% CI)	P Value†	OR (95% CI)	P Value†
First hemoglobin concentration, g/L				
≤115	1.7 (0.9-3.2)	.05	1.9 (0.9-3.8)	.02
116 to 125	0.9 (0.6-1.4)		0.9 (0.6-1.5)	
126 to 135‡	1.0		1.0	
136 to 145	1.3 (0.8-2.1)		1.5 (0.9-2.6)	
≥146	2.7 (1.1-6.4)		3.7 (1.4-10.0)	
Early pregnancy weekly change in hemoglobin concentration, g/L				
≤−1.6	0.8 (0.4-1.7)	.17	0.8 (0.4-1.8)	.46
−1.5 to −1.1‡	1.0		1.0	
−1.0 to −0.6	1.3 (0.7-2.1)		1.4 (0.8-2.5)	
−0.5 to −0.1	1.1 (0.7-1.9)		1.4 (0.7-2.4)	
0.0 to 0.4	1.8 (1.0-3.4)		1.8 (0.9-3.7)	
≥0.5	2.2 (1.0-4.9)		1.7 (0.7-4.2)	

*Adjusted for maternal age, height, occupation, smoking, body-mass index, and week of first hemoglobin concentration measurement; n indicates number of cases/controls; OR, odds ratio; and CI, confidence interval. First hemoglobin concentration is adjusted for early pregnancy weekly change in hemoglobin concentration and vice versa.
†Wald test for the overall effect (test of general heterogeneity).
‡Reference group.

gated primiparous women with singleton pregnancies, and the conclusions from this study can only be interpreted for this group. We were restricted to data found in the archive files and could not investigate other potential confounders, such as, nutritional intake, or physical activity. Furthermore, we could not study any additional hematological parameters besides hemoglobin concentration.

Iron supplementation improves maternal hematological indexes during pregnancy.[19] In Sweden the clinical guidelines recommend iron supplementation for pregnant women, unless hemoglobin concentration is high.[18] Thus, women with high hemoglobin concentration at the first visit for antenatal care are less likely to receive iron supplementation compared with those with a lower hemoglobin concentration. A recent review of clinical trials states that routine iron supplementation "had no detectable effect on any substantive measures of either maternal or fetal outcome."[20] In this study, we did not have information on iron supplementation and therefore could not investigate its possible confounding of the association between hemoglobin concentration and risk of stillbirth.

A U-shaped association between lowest hemoglobin concentration during pregnancy and stillbirth rates has been reported previously,[10] and 2 studies have reported increased rates of perinatal death with high- and low-hemoglobin concentration during pregnancy.[4,6] However, change in hemoglobin concentration during pregnancy was not considered in these studies. In our investigation, we adjusted for week of first hemoglobin measurement and used no hemoglobin measurements after stillbirth of the case or delivery of the individually matched control. Thus, bias due to measurements of hemoglobin concentrations at different gestational weeks for cases and controls was minimized.

Plasma volume expansion and lowered hemoglobin concentration are physiologic responses to pregnancy.[21] Plasma volume expansion is considered important for fetal growth,[22] and several studies have reported an increased incidence of low birth weight associated with a high maternal hemoglobin concentration.[5-7,9] The mechanism by which expansion of the plasma volume enhances fetal growth is not known, but reduced blood viscosity may favor blood flow in the maternal intervillous space.[9] High hemoglobin values are associated with placental infarction,[4] and pregnancy hemodilution may, by preventing thrombosis in the uteroplacental circulation, promote fetal nourishment and growth.[5] In our investigation, we used weekly change in hemoglobin concentration as an indirect measure of plasma volume expansion. However, weekly change in hemoglobin concentration was not significantly associated with stillbirth risk, and did not influence stillbirth risks related to first hemoglobin concentration. Moreover, although women with the highest initial hemoglobin value had the largest drop in hemoglobin concentration, the risk of stillbirth was almost exclusively confined to this group. Thus, high hemoglobin levels in early pregnancy may per se be deleterious for fetal survival.

Small for gestational age is associated with a high hemoglobin concentration during pregnancy,[6,9] and intrauterine growth restriction is one of the main determinants of stillbirth.[23,24] It is therefore noteworthy that the highest risks in our study were found for antepartum nonmalformed SGA stillbirths, suggesting that the risk of stillbirth related to high hemoglobin concentration measured during antenatal care is associated with impaired fetal growth. As fetal growth restriction is reported to be more important for preterm than term stillbirths,[25] the hemoglobin-related risk increase in preterm stillbirths may have a similar mechanism.

Hemoconcentration with hypovolemia is a feature of preeclampsia.[6,26] The reduction in plasma volume is reported to be proportional to the severity of the condition,[27] and the hematological changes occur before the onset of preeclampsia.[28,29] Because preeclampsia and eclampsia may lie in the causal path-

way between high hemoglobin concentration and stillbirth, we did not control for them in the multivariate analysis. However, when women with preeclampsia or eclampsia were excluded, the stillbirth-related risks due to a high hemoglobin concentration increased, suggesting that a high hemoglobin concentration primarily influences risk of stillbirth in nonpreeclamptic pregnancies.

The exact mechanisms for the association between maternal hemoglobin concentration and stillbirth, as well as the possible effects of iron supplementation, require further studies. Women with a high hemoglobin concentration at first measurement during antenatal care are reported to be at increased risk of SGA births,[6] and we found that a high first hemoglobin concentration increased the risk of stillbirth, especially antepartum-SGA stillbirths. We therefore suggest that pregnancies with high hemoglobin concentrations should be considered as high-risk pregnancies. To improve antenatal detection of fetal growth disturbances, it may be prudent to perform repeated ultrasound scannings on these pregnancies.

Author Contributions: Dr Stephansson contributed to the design of the study, was responsible for collection of the data, performed parts of the analyses, and took main responsibility in writing the manuscript.

Drs Dickman and Johansson performed the statistical analyses and assisted in the interpretation of the results and writing of the manuscript. Dr Cnattingius developed the study and assisted in the interpretation of the results and writing of the manuscript.

Funding/Support: This study was funded by grants from The Swedish Council for Social Research (project 98-0265:1B) and Karolinska Institutet.

REFERENCES

1. Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Association of maternal hematocrit with premature labor. *Am J Obstet Gynecol*. 1988;159:107-114.
2. Klebanoff MA, Shiono PH, Selby JV, Trachtenberg AI, Graubard BI. Anemia and spontaneous preterm birth. *Am J Obstet Gynecol*. 1991;164:59-63.
3. Scholl TO, Hediger ML, Fischer RL, Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. *Am J Clin Nutr*. 1992; 55:985-988.
4. Naeye RL. Placental infarction leading to fetal or neonatal death: a prospective study. *Obstet Gynecol*. 1977;50:583-588.
5. Sagen N, Nilsen ST, Kim HC, Bergsjo P, Koller O. Maternal hemoglobin concentration is closely related to birth weight in normal pregnancies. *Acta Obstet Gynecol Scand*. 1984;63:245-248.
6. Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. *Lancet*. 1986;1:992-995.
7. Huisman A, Aarnoudse JG. Increased 2nd trimester hemoglobin concentration in pregnancies later complicated by hypertension and growth retardation: early evidence of a reduced plasma volume. *Acta Obstet Gynecol Scand*. 1986;65:605-608.
8. Knottnerus JA, Delgado LR, Knipschild PG, Essed GG, Smits F. Haematologic parameters and pregnancy outcome: a prospective cohort study in the third trimester. *J Clin Epidemiol*. 1990;43:461-466.
9. Steer P, Alam MA, Wadsworth J, Welch A. Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. *BMJ*. 1995; 310:489-491.
10. Garn SM, Ridella SA, Petzold AS, Falkner F. Maternal hematologic levels and pregnancy outcomes. *Semin Perinatol*. 1981;5:155-162.
11. Cnattingius S, Ericson A, Gunnarskog J, Kallen B. A quality study of a medical birth registry. *Scand J Soc Med*. 1990;18:143-148.
12. Swedish Socioeconomic Classification. *Reports on Statistical Co-ordination*. Stockholm, Sweden: Statistics Sweden; 1995.
13. National High Blood Pressure Education Program Working Group. Report on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol*. 1990;163:1691-1712.
14. Centers for Disease Control. Criteria for anemia in children and childbearing-aged women. *MMWR Morb Mortal Wkly Rep*. 1989;38:400-404.
15. Winbo IG, Serenius FH, Dahlquist GG, Kallen BA. A computer-based method for cause of death classification in stillbirths and neonatal deaths. *Int J Epidemiol*. 1997;26:1298-1306.
16. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr*. 1996;85:843-848.
17. SAS Institute Inc. *SAS/STAT Software: Changes and Enhancements Through Release 6.12*. Cary, NC: SAS Institute Inc; 1997.
18. *Health Care Before, During and After Pregnancy* [in Swedish]. Stockholm, Sweden: National Board of Health and Welfare; 1997. Report 1996:7.
19. US Preventive Services Task Force. Routine iron supplementation during pregnancy: policy statement. *JAMA*. 1993;270:2846-2848.
20. Mahomed K. Iron supplementation in pregnancy [Cochrane Review on CD-ROM]. Oxford, England: Cochrane Library Update Software; 1999. Issue 4.
21. Whittaker PG, Macphail S, Lind T. Serial hematologic changes and pregnancy outcome. *Obstet Gynecol*. 1996;88:33-39.
22. Steer PJ. Maternal hemoglobin concentration and birth weight. *Am J Clin Nutr*. 2000;71(supp5):1285S-1287S.
23. Berkowitz GS, Papiernik E. Epidemiology of preterm birth. *Epidemiol Rev*. 1993;15:414-443.
24. Cnattingius S, Haglund B, Kramer MS. Differences in late fetal death rates in association with determinants of small for gestational age fetuses: population based cohort study. *BMJ*. 1998;316:1483-1487.
25. Gardosi J, Mul T, Mongelli M, Fagan D. Analysis of birthweight and gestational age in antepartum stillbirths. *Br J Obstet Gynaecol*. 1998;105:524-530.
26. Sagen N, Koller O, Haram K. Haemoconcentration in severe pre-eclampsia. *Br J Obstet Gynaecol*. 1982;89:802-805.
27. Chesley LC. Plasma and red cell volumes during pregnancy. *Am J Obstet Gynecol*. 1972;112:440-450.
28. Blekta M, Hlavaty V, Trnkova M, Bendl J, Bendova L, Chytil M. Volume of whole blood and absolute amount of serum proteins in the early stage of late toxemia of pregnancy. *Am J Obstet Gynecol*. 1970; 106:10-13.
29. Gallery ED, Hunyor SN, Gyory AZ. Plasma volume contraction: a significant factor in both pregnancy-associated hypertension (pre-eclampsia) and chronic hypertension in pregnancy. *QJM*. 1979; 48:593-602.

ORGANISATION FOR ECONOMIC
CO-OPERATION AND DEVELOPMENT

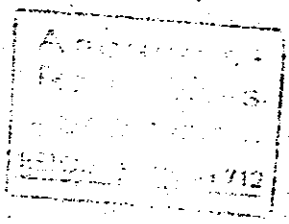
EUROPEAN NUCLEAR
ENERGY AGENCY

SEN/IR(65)15

RESTRICTED

Paris, 8th November 1965

Or. Engl.



STEERING COMMITTEE FOR NUCLEAR ENERGY

STUDY GROUP ON FOOD IRRADIATION

ON GENETIC EFFECTS PRODUCED BY IRRADIATED FOOD AND FOOD COMPONENTS

by

G.T. Scarascia-Mugnozza (Rome),
A.T. Natarajan and L. Ehrenberg (Stockholm)

On Genetic Effects Produced by Irradiated Food and Food Components.

G.T. Scarascia-Mugnozza (Rome), A.T. Natarajan and L. Ehrenberg (Stockholm).

1. Introduction.

An attempt has been made to summarize and evaluate available data on genetical effects (mutation and chromosomal aberration) induced in different organisms exposed to irradiated food or food components. Since published as well as unpublished work has been included the present report cannot be considered to be complete.

Experiments with plants and lower organisms are included because it may be stated generally that a substance which releases gene mutation in one organism is also mutagenic in all other organisms. If therefore an irradiated system (such as food or food component) is found mutagenic in e.g. higher plants, this effect should be expected to have a counterpart in mammals including man. A lower mutagenic efficiency in mammals of a principle active in microorganisms, plants, and lower animals may partly be due to unavailability of the gonads to compounds absorbed via the digestive tract or the lungs.

Positive and negative results have been obtained in different experiments even with the same organism. It is clear that enough data are not yet available for the evaluation of genetical hazards connected with human consumption of irradiated food, and the purpose of the present scientific report is not to do such an evaluation but to present the background for the further experiments needed.

2. UV effects.

It was shown relatively early that UV-irradiated media produce gene mutation in the bacterium Staphylococcus aureus[1] and in the sexually reproducing lower plant Neurospora crassa[2]. The same mutation spectrum as with direct irradiation of the organisms was obtained. The mutagenic radiomimetic) action could be ascribed to certain hydroperoxides (See summaries [3, 4]). - The UV-effects will not be discussed further in this context.

3. Ionizing radiations.

3.1. Microorganisms.

3.1.1. Irradiated solid glucose and protein showed when tested on lysogenic bacteria no increase in lysogeny;[5] although this system is very sensitive to direct irradiation.[6] The experiments are perhaps not conclusive because too low doses were used (1-5 Mrad to solid compounds, cf. Appendix).

3.1.2. Most work with microorganisms was done with UV-irradiated media (See 2). A slight increase of mutation to streptomycin-independent in *E. coli* has been observed after treatment with a distillate from irradiated animal standard food.[9] The effect, which was observed to some degree also in the control with irradiated food, is open to criticism because of the low pH during treatment which may cause mutation by depurination.

3.1.3. It may be expected that especially microorganisms will show increased mutation rates when treated with X- and γ-irradiated media under conditions giving certain (hydro)peroxides.[10,11] (Cf. 4).

3.2. Higher plants.

Most of the available data in higher plants are cytological, describing chromosomal aberrations in root meristems induced by irradiated media and other systems (3.2.1.-3.2.9.). Certain plant materials present a good system for the detection of and screening for chromosomal aberrations. It should be noted, however, that species of the family Leguminosae are sensitive to an effect of anaerobiosis during germination (but not later divisions) producing chromosomal aberrations.[12,13] Cereals do not show such effects and data from these plants are therefore more reliable.[14] The same holds true for the "Allium test".[15]

Only few efforts have been made to measure mutation rates (3.2.10.).

3.2.1. Early experiments of Natarajan and Swaminathan[16] demonstrated that X-irradiated distilled water can induce chromosome breaks in wheat and onion root tips (See Appendix).

3.2.2. In another experiment it was observed that chromosome breaks were produced if the cereal embryos were grown in X-irradiated Whites' cultural medium (for composition, see Appendix). These experiments were repeated later with γ-rays by Natarajan (unpublished) and the above results were confirmed.

3.2.3. Irradiated thymine solution was found also to induce chromosome breaks in barley.[14] There was a low frequency of breaks induced by unirradiated thymine solution, but the frequency was significantly higher with irradiated thymine solution (se Appendix).

3.2.4. Moutschen and Matagne[19] studied the effect of solutions of irradiated glucose monohydrate (Y-ray doses from 0.05 to 74 Mrad) on barley and Allium roots. An elaborate analysis was made in this study. A 6 fold increase in the chromosome aberration frequency, over the control, was observed. The interesting observations made in this study was the lack of any dose dependent increase of breaks, the highest rate (9-10 %) being reached with the glucose irradiated with 0.5 to 1 Mrad. (See Appendix). The dose independence of these indirect effects has been observed in Drosophila[20] and mice also. (See Discussion).

3.2.5. Very few studies have been conducted in this respect with food or food components. Swaminathan et al.[21] studied the effect of irradiated potato mash (20, 40, 80 kr) on barley root meristems by culturing barley embryos on the mash. A large number of cells was analysed in this study which indicated the presence of a high frequency of laggards and micronuclei probably due to chromosome fragmentation. (See Appendix). When the embryos were grown (on the irradiated medium) in the presence of oxygen, the effect was considerably reduced. Chopra and Swaminathan[22] irradiated potato tubers with 20 kr and stored them at +4°C får 8 months, and then they were meshed, and used as medium for the culturing of barley embryos. An appropriate control was kept with unirradiated potatoes. The developing root meristems showed a considerable increase in cytological aberrations (mostly of micronuclei).

3.2.6. Chopra et al.[23] studied the effect of irradiated fruit juices, on the root meristems of barley and Allium. There was a distinct and significant increase in the chromosome aberration frequency of the treated material in comparison to the controls. (See Appendix). The characteristics of the type of breaks induced was the localization at the centromeric region (resembling certain effects of alkylating agents) and the absence of exchanges.

3.2.7. Unpublished results of De Stanchina have indicated that irradiated orange juice induces only mitotic inhibition, but no chromosomal aberrations. (See Appendix).

3.2.8. Moutschen et al. (unpublished) have also tested the possible radiomimetic activity of (a) straw berry powder irradiated with 0.5 to 5 Krads of 3 MeV β-rays (b) cooked potato powder - irradiated with 10 Krad (1 Mev) (c) mashed potato treated with 20-80 Krad of X-rays (d) raw potato treated with 16-70 Krad of Y-rays (^{60}Co) and (e) wheat flour + soya flour exposed to a total dose of 200 Krad of Y-rays. All results were negative.

3.2.9. Although of less connection with the problem of irradiated foods, a few other experiments showing indirect radiation effects may bereported. D'Amato and Moletti (personal communication) transplanted unirradiated embryos over presoaked endosperm irradiated with low doses (0.5 to 1 Krad) and found that 24 hours after transplantation 20 to 25 % aberrant rot tip cells at anaphases contained chromatid breaks and deletions frequently, and bridges very rarely.

Kuzin[27], in a series of studies,has demonstrated the presence of nucleotoxic substances in extracts of irradiated plant tissues, capable of reducing mitotic index as well as induce chromosome aberrations in other tissues. The activity has been attributed to the formation of polyphenols.

3.2.10. Gene mutation.

Very few experiments have been conducted to demonstrate the possible mutagenicity of irradiated compounds in higher plants. Ehrenberg[28] studied the effect of irradiated glucose and glycine (50×10^6 R of ^{60}Co Y-rays), on barley seeds. The chlorophyll mutations were scored in the second generation, while the irradiated substrate gave a mutation rate of 1 %, the unirradiated substrate gave 0.4 %; the effect being significant at 0.02 % level.[x)]

3.3. Drosophila.

This insect represents one of the most officient genetic systems of the class of higher organisms. Several techniques for quantitative detection of mutation frequencies have been developed in this organism.

It may be mentioned that Kraybill suggested that Musca domestica, the house-fly, which affords similar possibilities, should be utilized in the evaluation of mutagenicity or carcinogenicity of degradative products arising from processing of food.[29]

In several experiments Drosophila has been fed with irradiated basic media (containing water, glucose, agar, yeast powder, and propionic acid) or with basic medium containing irradiated DNA.

x) When this experiment was repeated, no increase of mutation rate was obtained.

The results are contradictory, certain studies showing raised mutation rates, others no effect.

Chromosomal aberrations after feeding with irradiated medium were not studied in this material.

3.3.1. Swaminathan et al.[30] observed a 3 - 4 fold increase over the spontaneous rate of sex-linked recessive lethals after feeding with irradiated basic medium (150 Krad Y-radiation). The Muller - 5 technique was used following feeding of the wild type males from egg to adult stage. The males were then mated to females raised on unirradiated medium. The induced mutation is significant at $P < 0.01$ (cf. Appendix). Also visible mutants were registered

techniques.

3.3.2. Chopra[31] repeated the experiment of Swaminathan et al. (see 3.3.1), using X-chromosome recessive lethals. Certain differences were introduced, however, (a) a higher dose, (b) a changed composition of the medium (sucrose instead of glucose), (c) the media were not immediately used (transported from a far-off Y-source). No mutagenic effect was found. In view of the differences mentioned, in view of the smallness of the experiment, and in view of the fact that experimental details permitting the evaluation of clustering and, hence, a statistical treatment, the experiment does not contradict or invalidate the earlier[30] result. (See Appendix).

3.3.3. Moutschen and Moutschen-Dahmen[32] have fed both the OR-K and Muller-5 flies - with medium irradiated with 150 Krad, from 1 to 9 generations. The data gave a frequency of 18 cases out of 1366 (i.e. 1.32 %), in contrast to 3 cases in 1196 in the control (0.251 %). These data indicate a 5 fold increase in mutation rate. The statistical significance is high. (See Comments).

3.3.4. A detailed experiment, utilizing adequate statistical treatment, has been done by Rinehart and Ratty[20]. Flies were allowed to feed on an irradiated medium (irradiated with X-ray doses of 150, 500, and 3000 Krad). Two types of treatments were done: in one experiment, the medium was used immediately after irradiation and in another, the medium stored for 3 weeks after irradiation was used. There was a small but significant increase in the mutation frequency. (See Appendix). The same increase was obtained at all tested doses. The increase was found to be mainly from genial mutants (i.e.

increase of clusters of mutants) which was absent in the control, except in one case. In the series with immediate treatment there seems to be an increase in non-genial mutants, too (= post meiotic mutants). In the aged series there was a decrease in non-genial mutants, although it is indicated that genial mutation rates are still high.

3.3.5. It may be mentioned that Moutschen used the Muller-5 technique to determine the mutagenic effect of irradiated potatoes (8 Krad) and irradiated soya flour (200 Krad). No effect was found. Only 150-250 chromosomes were tested in each treatment. This size of the experiment is too small to detect mutation rates below around 5 %. The experiment is therefore not conclusive in any direction.

3.3.6. Irradiated DNA. On Parkash[33,34] published recently results indicating a strongly increased mutation rate following admixture of irradiated DNA to the food. The DNA (fish sperm) was irradiated with 10^5 rad and added to the medium, in which eggs were laid. When testing sex-linked recessive lethals[33] (CIB technique), 5.7 per cent mutation was reported against 0 in the control (representative for standard average = 0.17 %). The experiment was not designed to detect clusters, and the increase is therefore not significant (see Appendix). In a test of II chromosome autosomal recessive lethals[34] a similar increase was found. In this case a test for clustering was done (personal communication) indicating that a high mutation rate was really obtained.

Two laboratories (Cf. Chopra[31] and Rinehart and Ratty[35]) have repeated Parkash's experiment on sex-linked recessive lethals with addition of irradiated DNA to the medium. In none of the experiments any mutagenic effect is shown, and the experiments are big enough to exclude, with a high significance, even a two-fold increase of mutation. Chopra also considers the possibility of yeast counteracting the mutagenic effect of irradiated DNA. His material on that point is not big, but seems to exclude the high mutagenicity found by Parkash, also in the absence of yeast.

Nobody has repeated Parkash's experiments with II chromosome lethals.

Although without direct inference to effects of feeding irradiated food, it should also be recalled that Fahmy and Fahmy[36] failed to find lethals following injection of irradiated Drosophila

DNA, in the vicinity of the gonads of the flies. (In these experiments it was found, however, that DNA degraded by ultrasonics or irradiation and then injected is able to induce "minutes", i.e. small deletions which may be of genetical significance.

We may conclude from these experiments that irradiated DNA does not induce mutation generally (and that it certainly does not require special attention in the evaluation of the wholesomeness of irradiated food). The results of Parkash are scientifically very interesting, however, and a comparison of the DNA used in his tests with other products are recommended.

3.4. Mammals.

3.4.1. Data from multigeneration feeding tests. - The large-scale animal feeding tests performed in order to establish the wholesomeness of irradiated foods have not been designed in a way permitting an estimation of possible genetical hazards. It is true that no significant (or clearly accidental) effects have been found on the breeding of the animals in multigeneration tests (see e.g. reports on feeding tests with irradiated pork,[37] irradiated potatoes[38] and irradiated wheat and wheat products[39]). But the litter size may be affected by trivial dietal factors influencing e.g. the number of corpora lutea etc., and it is further a very insensitive measure of lethal mutation rate (cf. Lüning's[40] studies of dominant lethal mutations in irradiated mice). Therefore, the litter size and the breeding of the animals cannot be used for the estimation of mutation in mammals.

3.4.2. No studies of genetical effects in mammals are at present published, but a few cytological and also genetical experiments in mice of a more or less preliminary character have been run chiefly by J. and H. Moutschen-Dahmen, partly in collaboration with L. Bugyaki and A. Lafontaine.

3.4.3. Chromosomal aberrations in mice.

All experiments to be reported utilize spermatogonial divisions in mice as object of study. Studies of dividing lymphocytes in rats, under lymphoponic conditions (see 3.4.5.) produced by irradiated

conclusive these studies require a further development of the phytohemagglutinin technique for rat lymphocytes.

3.4.3.1. J. and M. Moutschen-Dahmen (unpublished) studied preliminarily in 5 exposed and 5 control mice the aberrations produced by irradiated "Hesby" food (for composition, see ref.[41]). The former exhibited an increased rate significant at P ≈ 0.05.

3.4.3.2. The same investigators assayed the influence of irradiated glucose, dissolved and given to the mice in the drinking water during 48 hours (somewhat arbitrarily chosen time, found to the suitable in corresponding tests with alkylating chemicals[42]). The glucose was irradiated as the monohydrate, i.e. the modification the radiation chemical products of which are most efficiantly killing bacteria[7]. The experiments were run as parallels to the botanical studies published (see 3.2.4.).

In the first series comprising irradiation with 5 Mrad and preparation of a 2 % solution the spermatogonial anaphase and the two spermatocyte anaphases were studied. In the five exposed animals 28 aberrant among 871 investigated cells were found, in comparison to 7 cells out of 800 in the five control animals given unirradiated glucose. The difference is strongly significant if we consider the aberrant cells independent (i.e., if no grouping to certain sensitive animals occurs).

In the second series/glucose irradiated with 74 Mrad was given under similar conditions. The summarized results (cf. Appendix) shows 34 aberrant out of 1235 studied cells in the five test animals compared to 3 aberrations in 1016 cells of the five control animals. Even in this case, and under conditions of independence of aberrant cells a high significance is obtained for the difference.

It may further be stated that in each of the divisions studied, the same increase of aberration rate was obtained in the two series, although the radiation dose of the latter was 15 times higher.

3.4.3.3. In cooperation with Bugyaki and Lafontaine a bigger experiment with 30 exposed and 30 control mice was run with irradiated food. 50% of a standard animal diet similar to the Hesby food was irradiated. The males were treated for 4 months from weaning. The results are summarized in Table 1, which also gives the aberration types observed. It appears that the frequencies of cells with aberrations are around 3 times higher in the animals exposed to irradiated food compared to the controls, and this increase is highly significant.

3.4.3.3. Table 1. General proportions of disturbed cells.

I. Animals fed irradiated food.

Metaphase I		Anaphase I		Anaphase II		Gonial anaphase	
Number of analysed cells	Number of disturbed cells	Number of analysed cells	Number of disturbed cells	Number of analysed cells	Number of disturbed cells	Number of analysed cells	Number of disturbed cells
1028	8	1212	33	1743	20	1962	33
II. Control animals.							
1028	1	1005	8	1273	6	1838	11

3.4.3.3. Table 2. Proportion of aberration types.

I. Animals fed irradiated food.[x)]

Metaphase I		Anaphase I		Anaphase II		Gonial anaphase	
Translo-cations	Other dis-turbances	Bridges	Fragments	Bridges	Fragments	Bridges	Fragments
7	1	25	18	9	13	16	20
II. Control animals.							
0	1	2	7	3	4	7	11

x) Numbers of analysed cell the same as in Table 1.

In order to evaluate the significance the statistics most disadvantageous to the experiment may be used assuming a maximum grouping of the aberrations to certain "abnormal" animals, restricting the analysis to animals with and without aberrant cells (cf. Table 3). Nearly all treated animals exhibit aberrations, in contrast to about half of the control animals. P = 0.0017 for the hypothesis that the frequencies of animals with aberrations are similar in the two groups.

3.4.3.4. Table 3.

Observation	Experiment with complete irradiated diet (3.4.3.3.)		Experiment with irradiated glucose (2nd series, 74 Krad, 3.4.3.2.)	
	Exposed animals	Control animals	Exposed animals	Control animals
Animals with no aberrations	3	14	0	4
Animals with aberrations	27	16	5	1
Of these, animals with translocations	5	0	2	0

The frequencies of animals exhibiting translocations (observed at metaphase) may be treated in the same way. In the treated series the 7 translocations found are distributed among 5 animals, compared to 0 such animals in the control series. P for equal distribution is about 0.03. To this series may be added the second glucose series, with 2 animals against 0 with translocations (cf. Appendix 3.4.3.2. Table 2). Testing the hypothesis that 7 out of 35 represent the same frequency as 0 out of 35, we find P = 0.006.

3.4.4. Mutation.

3.4.4.1. K.G. Lüning (unpublished) performed one experiment with mice where males fed irradiated food of the type demonstrated to produce a lymphopenia in rats.[41] Since the lymphopenia corresponded to that induced by about 30 rad total body γ-irradiation, the experiment was designed to detect the frequency of dominant lethal mutations induced by this dose in spermatids - sperms. The result was negative, the expected increase of the frequency of dead implantations not being found. (See Appendix).

3.4.4.2. J. and K. Moutschen-Dahmen have run a few preliminary experiments with different age of animals and different feeding periods. In large, the results agree with those of Lüning in the fact that, when males were fed the irradiated diet, no significant increase of decidua (per corpora lutea or per implantation) was found.

It was strongly indicated, however, that when both sexes were fed the irradiated food (the feeding of females with irradiated food was interrupted before gestation) the frequency of dead implants was increased by a factor 2-3. ($P \approx 0.01$ for equality of test series and control). (See Appendix).

We regret that this important work had to be discontinued for financial reasons at a moment certain interesting results started to appear, and a continuation of this type of work is strongly recommended.

In order to avoid misinterpretation we want to stress the extremely preliminary nature of Moutschen's data. Many factors of a more or less trivial nature may influence the shedding of eggs and their fertilization and early development, with a possibility to disturb the determination of dominant lethals. The number of corpora lutea per female seems thus to be affected by minor factors in the food, such as heating to 80° during the preparation of an

otherwise unchanged standard diet;[40] (hence, no conclusions as to absence or presence of lethal mutations should be drawn from an unchanged or decreased average litter size). It should also be mentioned that a control series (of a ^{90}Sr experiment) of mouse males receiving NaCl injections exhibited high frequencies of "dominant lethals" when mated within 3 weeks after injection; the reasons for this behavior is being investigated.[43]

It is extremely important to verify Moutschen's observation of increased number of decidua, with well comparable[x] controls and series where males, or females, or both sexes are fed irradiated diets during different periods of the life span. The intrauterine death observed after feeding both sexes may indicate a raised mutation rate, but it may also indicate a non-tolerable somatic damage during foetal development.

3.4.5. Although a leukopenia (lymphopenia) may be produced by many disturbances (see e.g. lists presented by Wintrobe[44], p. 1098) other than radiations, radiomimetic chemicals, and antimetabolites affecting the genetic material, this effect, which has been clearly established to be produced in the rat by irradiated food,[41] glucose,[45] etc., may be mentioned in this context. The lymphopenia is easily demonstrated in a few animals, and the experimental conditions, under which this effect demonstrated that <u>something</u> (with a possible relationship to genetical damage) had happened to the animals, were used in the genetical studies of the Moutschen-Dahmen team. Due to the great difficulties and high costs involved in the testing of mutagenicity in mammals (and, especially, if the mutation rates are low) we find it recommendable that the procedure, in certain experiments at least, should consist in the two steps mentioned

(a) establishment of optimum conditions for development of any "radiomimetic" effect;

(b) test of mutagenicity and chromosomal aberrations under this set of conditions.

If the test (b) is positive, it must of course be established

---

x) with respect to age, location in the room, earlier litters, etc.

4. Discussion.

4.1. Established experimental facts.

In certain well-investigated series it has been clearly demonstrated that irradiated food (media, compounds) are able to induce gene mutation (as established by genetical tests) and/or chromosomal aberrations in organisms belonging to different classes: microorganisms, plants, insects, mammals.

The effects are mostly obtained in the range of doses, 10^5 - $5 \cdot 10^6$ rad, intended for radiopasteurisation and radiosterilisation.

In all investigated materials the frequencies of genetical changes are low - in many cases we are dealing with an increase by a factor 2-3.

In certain cases no effect was found. Such negative results were partly obtained under experimental conditions very similar to these giving positive results. It is most probable that the negative results do not invalidate the positive ones and v.v. We should rather draw the conclusion that still unknown variables in the test organism or in the food irradiated may affect the reaction pattern with the consequence that genetical damage is either induced or not induced. This increases the difficulties of analysis, but may be guiding in efforts to unravel the action mechanisms.

In a few cases the influence of radiation dose was investigated:

Drosophila, medium	0.15 - 3 Mrad
Mouse, crystalline glucose	5 and 74 Krad
Hordeum roots, glucose	0.05 - 74 Krad

In each case all the investigated doses give approximately the same mutation (Drosophila) or aberration (mouse, Hordeum) rate, respectively.

4.2. Action mechanisms.

The nature of the radiations used (mostly therapy X-rays or ^{60}

effects observed will therefore be due to radiation chemical changes in the irradiated food.

The absence of dose dependence in any case where the dose dependence was investigated, indicates that we are not dealing with principles with a reactivity vis-a-vis the gene material like

most radiomimetic alkylating chemicals. It is known that certain radiation chemical products, such as H_2O_2, may reach a steady state concentration maintained in a broad range of concentrations, but the possibility that reactive hydroperoxides are involved is less likely, partly in view of the fact that such compounds are not formed when irradiated crystalline glucose is dissolved in water (G. Löfroth and L. Ehrenberg, unpublished).

The absence of dose dependence (and partly the fact that no genetical change was found in certain cases) indicates that the genetical effects we observe are induced in an all-or-none response to a trigger mechanism, which is released when certain active compounds exceed a threshold dose. The degree of response will then be predetermined by physiological properties of the cell population exposed to the active compounds.

It is true that also a lack of an essential vitamin etc. may produce a physiological-biochemical change of the kind required for a changed mutation rate. In the animal experiments described such an explanation is less plausible, however, because only a fraction of the total, supervitaminized diet was irradiated. It has further been shown that other biological effects can be released by compounds which can be extracted from irradiated glucose solution[8] and irradiated animal diet.[9]

The dose-independent all-or-none character of the genetical effects resembles the properties of the rat lymphopenia induced.[41,9] In this case a possible "trigger" is indicated: The effect may partly at least be restored by folic acid given in the drinking water.

A direct or indirect anti-folic acid effect of irradiated foods would disturb the synthesis of thymidylate, i.e. a DNA precursor, in a way which might give chromosomal aberrations (as shown by Taylor for aminopterin[46]) or gene mutation (as shown by Heslot[47] to be induced in yeast by aminopterin and metathrexate). It should be observed that other investigators have found folic acid antagonists to be inactive in this respect.[48,49]

As another guide-line for further research it should be recalled that vegetable oils are able to produce chromosomal aberrations and gene mutation in higher plants.[50] This effect is exaggerated in methyl linolate[51], i.e. an unsaturated compound separated from the normal antioxidants. Since ionizing radiations

tend to destroy natural antioxidants, the possible consequences of this should be observed. (Compare with the fact that irradiation of oils - at high doses - has been demonstrated to induce a considerable toxicity to test animals[52]).

4.3. Possible consequences to human consumption of irradiated food.

The data presented in this report do not justify us to judge the genetical consequences to man of a general use of radiation preserved food. (The same concerns, of course, animals fed irradiated fodder.) At the present state of affairs genetical effects can be neither proved nor excluded.

In several instants a 2-3 fold increase in mutation rate in a broad sense, was observed to be induced, independently of the dose, in Drosophila and mice, and such an increase of mutation rate in large human populations would not be tolerated according to current principles for radiological protection. ICRP sets the "maximum permissible genetic dose",[53] given to members of the public, to 5 rem, a dose expected to increase the mutation rate by 5-50 per cent.[54]

It should be noted, however, that no food of a type intended for human consumption was tested in any of the animals (although it has been indicated that related effects in plants are produced by irradiated fruit juices, potatoes, etc.). We do not think that such tests are yet possible, e.g. with mice. The development of reliable techniques is dependent on further work with those food types, i.e. standard animal diets, which have already given certain genetical effects. These experiments must be repeated, and a series of essential variables must be evaluated.

We should further note that only the Drosophila experiments prove that gene mutation sensu strictu is induced by irradiated media. The chromosomal aberrations found in plants and mice are mostly of types eliminated before they can reach the following generation; in plants, certain aberrations have been demonstrated to be transferred, however, and this will probably, when investigated, also be the case in mammals.

It may also be said about the dominant lethal mutations, measured as intrauterine death, that if the effect is restricted to such early foetal death, the harm will be limited.

The genetical changes studied represent such effects which are most easily studied, and they do not coincide directly with

such more harmful effects the frequencies of which we want to estimate. It may be said, however, that most agents inducing any of the effects studied give rise to all other genetical effects including the most dangerous ones, and we have to accept this as a working hypothesis also for the irradiated foods until we have proved the spectrum of effects to be undangerous. If, as suggested, antimetabolic action mechanisms are involved, a very skew spectrum of effects would not be unexpected, however; (antimetabolites like nebularine and 8-ethoxycaffeine are known to induce restrictedly either gene mutation or chromosomal aberrations, respectively.[55])

The high frequencies of intrauterine death found in one experiment to follow feeding irradiated food to females, may as well be a measure of somatic damage to the foetus. If this damage includes types where the children are born alive a non-genetical but nevertheless very harmful effect may be indicated. From the American multigeneration tests with irradiated foods no increase of living, abnormal offspring was reported; this would indicate that such somatic damage is rare. (When such somatic effects are estimated it is important to chose the right test animals, however, as demonstrated by recent experience from tests with pharmaceuticals.)

5. Conclusions and proposals.

From the experiments herein reported it is impossible to arrive at any definite conclusion as to the presence or absence of genetic effects if irradiated food were used for human consumption or for animal feeding. Hitherto available data indicate, however, that increased rates of mutation and chromosomal aberrations will probably be induced in certain cases. Although experiments indicate that the genetical effect, in cases where it is induced, is relatively small compared to the effect of direct exposure of mammals to radiation, the same experiments indicate that the possible effect will not be negligible. The intrauterine death described may indicate lethal mutations or somatic damage to the foetus. Although non-genetical in principle the latter

For this reason new experiments with careful layout and strict coordination are needed. Especially when different laboratories obtain contradictory results, exchange of food samples and test organisms is recommended.

5.1. Since cytological aberrations, as chromosome breaks and translocations, are events implying a mutagenic effect, because of the possible formation of clones of mutated cells in the somatic tissues or in the gonads, the quick tests on chromosomes of higher plants are still required. Root meristems of cereal seedlings or onion bulbs can be employed. Cytological analyses should be performed in a way permitting the determination of not only the possible damage (absence of mitoses, chromosome breakage and rejoining) but also of the nuclear stage in which the possible action took place.

5.2. Relatively quick proofs of genetic effects can be obtained using microorganisms and lower animals, as Drosophila. In any case, the investigations must be carried out with different species. The analyses proposed under the items 5.1. and 5.2. seem useful and necessary; they complement each other. Basic differences, in fact, exist between the biological systems involved.

5.3. Experiments with "in vitro" tissue cultures should be carried out along with the development of this technique.

5.4. Undoubtedly, the definitive genetic approach, the results of which can be rather correctly extrapolated to man, has to be performed on different species of small mammals. Because of the complexity, cost and duration of such investigations, it might be convenient first to develop the techniques and clarify the influence of essential variables in well-planned experiments with those irradiated standard diets which induce chromosomal aberrations in mouse spermatogonia and possibly also intrauterine death in the same material. Tests with other foods should be done after they have been found effective in microorganisms, higher plants, or Drosophila, or under conditions giving a lymphopenia in rats.

5.5. If feeding tests with human volunteers (of the type already conducted in the US studies) are again started, indications of genetical changes in the test persons may be obtained from a study of chromosomal aberrations in phytohemagglutinin stimulated lymphocytes. For this purpose the background values (and X-ray pre-history, etc.) of the test persons should be determined in advance. The result of such a test should be judged in view of the ratio, which should be determined, of lymphocyte aberrations to gene mutation in animals.

5.6. Chemical and biochemical analyses of irradiated foods or food components might denounce the presence of formed noxious compounds. Their mutagenicity should be tested with the above mentioned biological systems. Genetical tests should thus be done with glyoxal, the factor identified to be responsible for the cytotoxic effect on human cells of irradiated glucose solution,[8] and also with active fractions from the irradiated standard animal diet which induce lymphopenia.[9] So also 3.2.9.

It seems important to find whether active principles responsible for the genetical effects of irradiated foods can be isolated and identified. In this chemical work rapid techniques (e.g. mutation in bacteria) must be used as guides to test the activities of fractions.

When, in one irradiated food, the factor responsible for genetical effects in one test organism has been identified, further work may be aided by the analysis for this and related compounds in other types of irradiated food.

5.7. In the evaluation of the genetical hazards from direct exposure of human beings to ionizing radiations, the mutation rates in test animals (mostly mice) are determined at high doses, and permissible doses are estimated by extrapolation. If we are dealing with, say, a 100 per cent increase of the mutation rate (to recessive characters) at all doses given to a food, the determination of this increase would require work with of the order of 10^5 animals. (Such a study has, in fact been started by Swaminathan et al., using Russel's mouse strain[56] with 7 recessive markers).

We feel that the enormous costs involved in such tests will require either that we accept data obtained in work with chromosomal aberrations and intrauterine death, or that we develop special, more efficient techniques to estimate low mutation rates, as has been done in higher plants.[57,58]

References
(to Text and Appendixes)

1. Stone, W.S., Wyss, O., and Haas, F. (1947) The production of mutations in Staphylococcus aureus by irradiation of the substrate. - Proc. Natl. Acad. Sci. 34, 142-149.
2. Wagner, R.P., Haddox, C.H., Fuerst, E., and Stone, W.S. (1950) The effect of irradiated medium, cyanide, and peroxide on the mutation rate in Neurospora. - Genetics 35, 237-248.
3. Stone, W.S. (1955) Indirect effects of radiation on genetic material. - Brookhaven Symposia in Biology 8, 171-190.
4. Zelle, M.R., and Hollander, A. (1955) Effects of radiations on bacteria. - Radiation Biology II: (Ed. A. Hollander, Mc Graw Hill), 365-430.
5. Bugyaki, P.L., Lafontaine, A., Moutschen-Dahmen, J. et M. (1963) Les aliments irradiés exercent ils un effect radiomimétique. 1. - Atompraxis 9, 1-3.
6. Marcovich, H. (1957) Etude radiobiologique du système lysogene d'Escherichia coli K-12. - Thesis, University of Paris, pp. 1-134.
7. Molin, N., and Ehrenberg, L. (1964) Antibacterial action of irradiated glucose. - Int. J. Rad. Biol. 8, 223-231.
8. Berry, R.J., Hills, P.R., and Trillwood, W. (1965) Demonstration of a cytotoxic agent in solutions of carbohydrates sterilised by ionizing radiation. - (Manuscript).
9. Löfroth, G., Hanngren, K., Ehrenberg, L., and Ehrenberg, A. (1965) Biological effects of irradiated food. II. Chemical and biological studies of compounds distilled from irradiated food. - Ark. Zool. (in press).
10. Kotin, P., and Falk, H.L. (1963) Organic peroxides, hydrogen peroxide, epoxides and Neoplasia. - Rad. Research Suppl. 3, 193-211.
11. Sobels, F.H. (1963) Peroxides, and the induction of mutations by X-rays, ultraviolet, and formaldehyde. - Rad. Research Suppl. 31, 171-183.
12. Levan, A., and Lotfy, T. (1950) Spontaneous chromosome fragmentatio in seedlings of Vicia faba. - Hereditas 36, 470.

13. Rieger, R., and Michaelis, A. (1958) Cytologische und Stoffwechselphysiologische Untersuchungen am aktiven Meristom der Wurzelspitze von Vicia faba L. I. Der Einfluss der Unterwasser-Quellung der Samen auf die chromosomale Aberrations-rate. - Chromosoma 9, 238-257.

14. Natarajan, A.T. (1960) Stored energy and indirect biological effects of radiations. - Risö Report 16, 39-40.

15. Levan, A. (1951) Chemically induced chromosome reactions in Allium cepa och Vicia faba. - Cold Spring Harbour Symp. Quant. Biol. 16, 233-243.

16. Natarajan, A.T., and Swaminathan, M.S. (1958) Indirect effects of radiations and chromosome breaks. - Ind. J. Genet. 18, 220-223.

17. Scholes, G., Weiss, T., and Wheeler, C.M. (1956) Formation of hydroperoxides from nucleic acids by irradiation with X-rays in aqueous systems. - Nature 178, 157.

18. Chevallier, M.R., and Luzatti, D. (1960) Action mutagène spécifique de trois peroxydes organiques sur les mutations reversis de deux loci de E. coli T-9-13. - Compt. rend. acad. sci. 250, 1572-1574.

19. Moutschen, J,. and Matagne, R. (1965) Cytological effects of irradiated glucose. - Rad. Botany 5, 23-28.

20. Rinehart, R.M., and Ratty, F.J. (1965) Mutation in Drosophila melanogaster cultured on irradiated food. - Genetics (in press)

21. Swaminathan, M.S., Chopra, V.L., and Bhaskaran, S. (1962) Cytological aberrations observed in barley embryos cultured in irradiated potato mash. - Rad. Research 16, 182-188.

22. Chopra, V.L., and Swaminathan, M.S. (1963) Sprout inhibition and radiomimetic properties in irradiated potatoes. - Die Naturwissenschaften 50, 374-375.

23. Chopra, V.L., Natarajan, A.T., and Swaminathan, M.S. (1963) Cytological effects observed in plant material grown on irradiated fruit juices. - Rad. Botany 3, 1-6.

24. Moutschen, J., and Moutschen-Dahmen, M. (1958) L'action du Myleran (Di-méthane-sulfonyloxy-butane) sur les chromosomes chez Hordeum sativum et chez Vicia faba. - Hereditas 44, 415-446.

25. Swaminathan, M.S., Chopra, V.L., and Bhaskaran, S. (1962) Chromosome aberrations and the frequency and spectrum of mutations induced by /ethyl methanesulfonate in barley and wheat. - Ind. J. Genet. 22, 192-207.

26. Natarajan, A.T., and Upadhya, M.D. (1964) Localized chromosome breakade induced by ethyl methanesulfonate and hydroxylamine in Vicia faba. - Chromosoma 15, 156-169.

27. Kuzin, A.M. (1962) On the role of the disturbance of metabolic processes in the radiation damage of the cell. - Int. J. Rad. Biol. 6, 211-220.

28. Ehrenberg, L. (1960) Chemical mutagenesis. Biochemical and chemical points of view of mechanisms of action. - Abh. Deut. Akad. Wiss. Berlin, Kl. Med. Jahr. 1960 Nr 1, 124-136.

29. Kraybill, H.F. (1962) General methods and problems in assesment of potential toxicity and carcinogenicity in Radiation and thermal processed foods and food additives. - Report of the FAO/WHO/IAEA Meeting on the Wholesomeness of Irradiated Foods. Brussels, 112-122.

30. Swaminathan, M.S., Nirula, S., Natarajan, A.T., and Sharma, R.P. (1963) Mutations incidence in Drosophila melanogaster reared on irradiated medium. - Science 141, 637-638.

31. Chopra, V.L. (1965) Tests on Drosophila for the production of mutations by irradiated medium or irradiated DNA. - Nature (in press).

32. Moutschen, J., and Moutschen-Dahmen, M. (unpublished).

33. Parkash, Om (1965) Induction of sex linked recessive lethals and visible mutations by feeding X-irradiated DNA to Drosophila melanogaster. - Nature 205, 312-313.

34. Parkash, Om (1965) On the radiomimetic effect of irradiated desoxy ribonecleic acid (DNA_m) on Drosophila melanogaster. - Die Naturwissenschaften 6, 142.

35. Rinehart, R.R., and Ratty, F.J. - Personal Communication.

36. Fahmy, O.G., and Fahmy, M.J. (1965) Genetic properties of exogenous deoxy ribonucleic acid at various levels of degradation in Drosophila melanogaster. - Nature 207, 507-510.

37. Bubl, E.C., and Butts, J.S. (1960) The growth, breeding and longevity of rats fed irradiated or non irradiated pork. - J. Nutrition 70, 211-218.

38. Okoumeva, L.A. (1958) Valeur hygiénique de la pomme la pomme de terre irradiée par le cobalt radioctif pour arrêter la germination. - Vopr. Pitania, S.S.S.R. 17, 49-53.

39. Brownell, L.E., Horne, T. and Kretlow, W.J. (1962) Petition for the use of gamma radiation to process wheat and wheat products for the control of insect infestation. (miniografed manuscript)

40. Lüning, K.G. (1964) Studies of irradiated mouse populations III. Accumulation of recessive lethals. - Mutation Research 1, 86-98.

41. Ehrenberg, L., Löfroth, G., and Ehrenberg, A. (1965) Biological effects of irradiated food. I. Effect on lymphocyte numbers in the peripheral blood of the rat. - Arkiv. Zool. (in press).
See also
Ehrenberg, L. (1962) The concept of stored energy (with special regard to radiomimetic effects produced by irradiated foods).- Report of the FAO/WHO/IAEA Meeting on the Wholesomeness of Irradiated Foods. Brussels, 166-178.

42. Moutschen, J. (1961) Differential sensitivity of mouse spermatogenesis to alkylating agents. - Genetics 46, 291-299.

43. Lüning, K.G. et al. (unpublished).

44. Wintrobe, M.M. (1961) Clinical hematology. - Henry Kimpton, (London).

45. Ehrenberg, L., and von Ehrenstein, G. (1960) How should a possible carcinogenic action of irradiated food be measured? - Risö Report 16, 41-43.

46. Taylor, J.H. (1963) DNA synthesis in relation to chromosome roproduction and the reunion of breaks. - Symp. Macromolecular Aspects of the Cell Cycle: J. Cell. Comp. Physiol. 62, 73-86.

47. Heslot, H. (1960) Note sur l'activité mutagène de certains antifoliques. - Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin. Jahrgang 1960, 1, 8.

48. Moutschen, J. (unpublished).

49. 1.Truhaut, R., and Deysson, G. (1963) Caractéristiques de l'activité antimitotique de l'aminopterine sur le test Allium. - C.R. Soc. Biol. (Paris) 157, 1963-1965.
2.Deysson, G., and Truhaut, R. (1963) Influence de l'acido folique et de l'acide folinique sur la cytotoxicité de l'aminopterine: étudos sur le test Allium. - C.R. Soc. Biol. (Paris) 157, 2179-2182.

50. Swaminathan, M.S., and Natarajan, A.T. (1959) Cytological and genetic changes induced by vegetable oils in Triticum. - J. Heredity 50, 170-187.

51. Natarajan, A.T., and Ehrenborg, L. (unpublished).

52. Lang, K. (1962) Studies on the wholesomeness of irradiated fats.- Report of the FAO/WHO/IAEA Meeting on the Wholesomeness of Irradiated Foods. Brussels, 148-156.

53. I.C.R.P. Publication 6. - Pergamon Press, 1964.

54. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation (1958) - General Assembly, Official Records, 13th session, Suppl. No. 17 (A/3838).

55. Ehrenberg, L., Gustafsson, Å., and Lundqvist, U. (1956) Chemically induced mutation and sterility in barley. - Acta Chem. Scand. 10, 492-494.

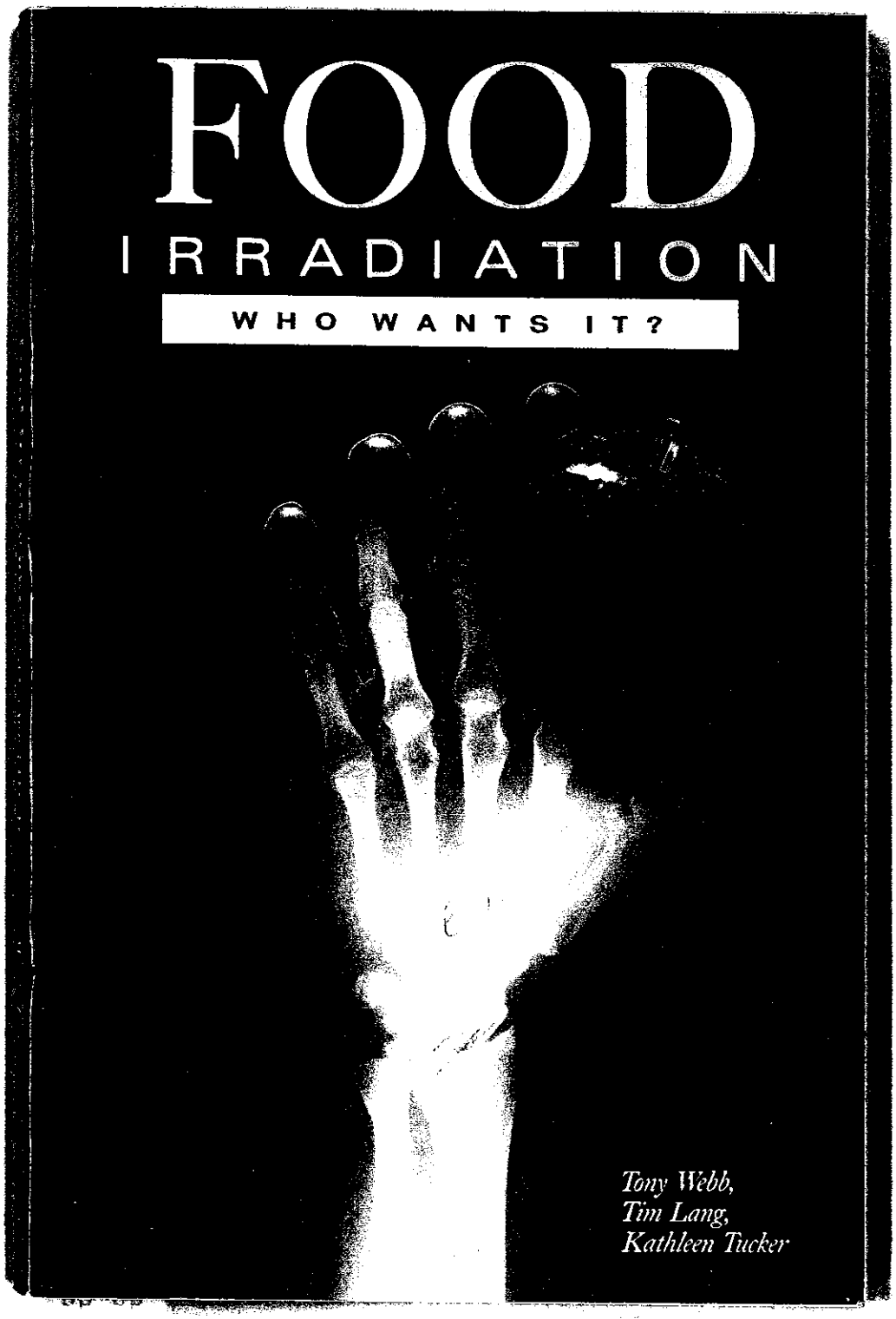
56. Russel, W.L. (1954) Genetic effects of radiation in Mammals. - Radiation Biology (Ed. A. Hollander, Mc Graw Hill) 1, 825-860.

57. Eriksson, G. (1963) Induction of waxy mutants in maize by acute and chronic gamma irradiation. - Hereditas 50, 161-178.

58. Ehrenberg, L., and Eriksson, G. (1964) Mutation in the waxy character in barley pollen grains following ^{90}Sr-incorporation at low activities. - Mutation Research, 1, 139-145.

FOOD IRRADIATION

WHO WANTS IT?



Tony Webb,
Tim Lang,
Kathleen Tucker

First published in Great Britain under the title Food Irradiation: The Facts by Thorsons Publishing Group, Wellingborough, Northhamptonshire, 1987.

Library of Congress Cataloging-in-Publication Data
Webb, Tony
Food irradiation.

Bibliography: p.
Includes index.
1. Food, Irradiated. 2. Radiation preservation of food. I. Lang, Tim. II. Tucker, Kathleen. III. Title.
TX571.R3W43 1987 363.1'92 87-10118
ISBN 0-7225-1071-3

Printed and bound in the United States

10 9 8 7 6 5 4 3 2 1

Distributed to the book trade in the United States by Harper and Row

Distributed to the book trade in Canada by Book Center, Inc., Montreal, Quebec

Distributed to the health food trade in Canada by Alive Books, Toronto and Vancouver

Distributed to the book trade outside the United States and Canada by Thorsons Publishing Group, Wellingborough, Northamptonshire, England

CHAPTER 4

WHOLESOMENESS OF IRRADIATED FOOD?

IN THE ENGLISH language, "wholesomeness" is a word that combines the ideas of safe, nourishing, and health-promoting. Unfortunately, there is no similar word in other languages, which means that in the international debate about food irradiation, the word has been debased so that it only covers the absence of any harmful effects. As long as there are no harmful toxic chemicals, or the microbiological and nutritional changes in the food do not cause obvious problems, the various scientists feel able to declare irradiated food "wholesome."

This narrow approach ignores questions concerning damage to essential constituents of food, such as vitamins and polyunsaturated fatty acids. It also ignores the effect that the technology may have on dietary health, particularly for people whose diet is already deficient. It also dismisses the impact of the technology on the natural balance of microorganisms that make up the ecology of our food.

It may be possible to show that irradiation, properly controlled, does not produce particular harmful agents, or that the risk of such events is slight and likely to be acceptable. Even so, the term "wholesome" cannot be applied to foods that may still appear fresh but that have been significantly denatured, either by the process of irradiation or by the extended storage times that irradiation makes possible. There is a big difference between "safe for human consumption" and "wholesome." This is a distinction the public can and does make, and which they need to be able to continue to make as a fundamental right. Wholesomeness, and the thorny question of the labeling of irradiated foods so that the consumer is made aware of the changed nature of the food, are issues that cannot be separated.

The issue goes deeper than protecting the individual's right to

know what is being done to food. We also need to consider the effects on public health of changes in the general diet that result when a significant portion of the diet comes from irradiated foods.

Public health agencies and nutritionists are beginning to get across the message that we need significant changes in the North American diet if we are to tackle some of the major causes of ill health, not least the heavy toll of coronary heart disease.[1] The same is true for most of Europe.[2] It is widely recognized that we need to cut down on the amount of fat and, within the remaining fat content of the diet, to substitute unsaturated fats for some of the saturated fats. We are also being encouraged to eat less red meat and to substitute white meats, such as fish and chicken, and some vegetable proteins, such as nuts and beans. We are being encouraged to eat more whole grains in bread, wheat, corn, and rice; to eat more fresh fruit and vegetables and to eat more of these uncooked. A healthy balance is developing around these ideas. The changes are taking place gradually and are no longer seen as the ideas of an affluent minority in the "health food" lobby. They are affecting the whole population as food processors and retailers realize that there is a widespread public demand for such changes. They have been helped in this by the actions of some school boards, as in New York, where public policy has actively promoted such changes with what have been suggested are improvements in pupils' health and scholastic achievement.

Yet, in the debate about the introduction of food irradiation, there has been hardly any consideration of the impact of the process on the diet. The very foods we are being encouraged to eat more of—white meats such as chicken and fish, whole grains, and fresh fruit and vegetables—are the target foods for irradiation, and all of them could suffer losses in some essential nutrients as a result.

Vitamins

It is not in dispute that irradiation causes damage to many vitamins. Vitamin A, vitamins B_1 (thiamine), B_2, B_3, B_6, B_{12}, folic acid, vitamin C (ascorbic acid), and vitamins E and K are all damaged to a greater or lesser extent by irradiation (Table 8). Essential polyunsaturated fatty acids (PUFAs) are also affected. These are increasingly being valued for their contribution to health

Table 8: Some Reported Percentage Vitamin Losses From Irradiation

Food	Vitamin A	B_1	B_2	B_3	B_6	B_{12}	C	E
Milk	60-70	35-85	24-74	33	15-21	31-33		40-60
Butter	51-78							
Cheese	32-47							
Grains and flour								
Wheat	—	20-63	—	15	3	—	—	—
Oats		35-86						7-45
Rice		22						
Beans	—	—	48	—	48	—	—	—
Meats								
Beef	43-76*	42*-84*	8-17*	—	21*-25*	—	—	—
Pork and ham	18*	96*	2*	15*	10-45*			
Chicken	53-95*	46-93*	35*-38*		32*-37*			
Eggs	—	24-61*	—	18	—	—	—	17
Fish								
Cod	—	47	2*	—	—	—	—	—
Haddock		70*-90*	4*		26			
Mackerel		15-85*						
Shrimp	2-27	70*-90*						
Potatoes	—	—	—	—	—	—	28-56	—
Fruits								
Fruit juices	—	—	—	—	—	—	20-70	—
Nuts	—	—	—	—	—	—	—	19-32

Note some vitamins are relatively undamaged by radiation, but absence of a figure in the table above does not imply that a food has been cleared. In general, more work needs to be done on a comprehensive study of vitamin losses.
*All losses are at doses below the 10 kGy proposed clearance level unless *. In these cases, doses are below the 60 kGy being suggested for sterilization of meat products
(Sources: References 13, 14, 15.)

as well as for the general benefits of substituting unsaturated for saturated fats. Vitamin E, which is known to be a protector of the PUFAs, is so badly damaged that in many cases it is even destroyed if it is put back into the food as an additive.

The extent of the loss depends on the vitamin, on the type of food, and on the dose of radiation given. Generally speaking, the more complex the food, the less it suffers vitamin losses during irradiation. Fruit juices suffer more than fresh fruits, and fruits more than vegetables, grains, and meat products. Nevertheless, as the table shows, losses of 20 to 80% are not uncommon, and there are still many gaps in the available scientific data on vitamin losses.[14,15,16]

Public Health Implications?

Folic acid (folate) is an essential vitamin from the group of B vitamins. In its brief and inadequate discussion of the issue, the British ACINF said that "little is known about the effects of irradiation on folate." Since there are possible problems in the area of public health in relation to the intake of folic acid, this needs further investigation. Deficiencies in folic acid and in other vitamins[3] and minerals[4] have been linked to the development of neural tube defects such as spina bifida. In addition, studies of elderly people and people entering mental hospitals have found that a significant proportion of such people have deficiencies in folic acid.

The United States is unique in having high recommended daily allowances (RDAs) for most vitamins. The European RDA for vitamin C is about half that in the United States, mainly because the average daily intake in northern Europe does not meet the United States standard. In Britain, a recent report from the Department of Health and Social Security found major deficiencies in the diet of British schoolchildren.[5] In many parts of the United States, particularly among those whose diet is limited by lack of adequate income, there are groups of people whose diet is already deficient.[6]

The same would apply to a much greater extent in less developed countries. Unfortunately, food irradiation advocates see nothing but benefits from irradiation in the war on hunger and malnutrition. The dream rapidly turns into a nightmare if we think that irradiation may be used to extend the storage life of the developed world's mountains of surplus food, so that these nutritionally depleted stockpiles can be off-loaded onto third world countries—adding insult to injury by calling it "aid."

The fact is that this technology has been introduced with too little consideration of the impact it will have on diet. No studies anywhere have assessed whether there will be a significant impact either on the population as a whole or on vulnerable groups within the population.

The first response of many of the experts who have been asked

to comment on this issue has been to argue that these losses are not likely to be significant. When the extent of the losses is pointed out to them, they say that it is within the normal range of losses that would occur naturally in cooking and storage. Since these normal losses vary between 0 and 100%, this is hardly surprising, but, in fact, the comparison is both untrue and misleading.

For example:

- Some irradiated foods are to be sold as fresh and, in some cases such as fruits and some vegetables, eaten fresh and raw. Consumers may assume they are getting full nutritional value when they are actually eating a vitamin-depleted processed food.
- Some vitamins, B_1 for example, undergo accelerated losses in storage if the food has been irradiated. As we noted earlier, vitamin E is virtually destroyed even if put back in as an additive.
- Irradiated food is intended to be stored longer, so nutrient storage losses will be greater.
- Irradiation and storage losses are then added to by losses in cooking and storing. Typical vitamin losses during household preparation of food are already significant, as Table 9 shows.

Table 9: Average Vitamin Losses During Household Preparation of Foods (%)

Thiamin	30
Riboflavin	15
Niacin	20
Ascorbic acid	35
Folic acid	40-50

(Source: Reference 16.)

The food can thus undergo initial losses on irradiation, accelerated losses during storage, and additional losses because of longer storage times, and then lose further vitamins in cooking.

In Britain, where there has been a furious debate on the issue of vitamin losses, the irradiation lobby has used a fall-back argument when confronted with these facts. Sir Arnold Burgen, the

chairman of the British ACINF, said that it would not be a problem because no one was likely to eat a significant portion of the total diet from irradiated foods.[7] We believe this comes dangerously close to saying that irradiated food is all right as long as you don't eat it!

At an earlier meeting with the British Food and Drink Federation, an industry spokesperson challenged us to name any food that was likely to be both irradiated and consumed in significant quantity. We suggested that the British were known world-wide for their consumption of fish and chips—and there was a rapid change of subject: Given that irradiation is considered appropriate for some fresh fruits and for potatoes, fish, and chicken, it is clear that some staple foods could be affected.

In a final attempt to defuse the public outcry, the British ACINF suggested that there should be long-term monitoring of irradiated food for nutritional damage. It would, we suggest, be better for such research to be done *before* the widespread application of irradiation to food, not after. As with the area of food safety, the opinions of the expert committee are no substitute for scientific evidence. It should also be noted that many of the adverse effects noted in the chapter on food safety could be caused by vitamin deficiencies in the diet of the experimental animals. In the absence of hard evidence on nutritional changes, the widespread consumption of irradiated food would be an uncontrolled human experiment.

Counterfeiting Freshness?

Irradiated foods look fresh longer. The consumer will be encouraged to view them as healthy and wholesome, whereas they are likely to be older and more depleted in essential health constituents. In these circumstances, the potential for deceit—for what can best be described as "counterfeiting" fresh food—is considerable.

In many consumer surveys that have been done around the world,[8,9,10,11] people have consistently demanded that irradiated food be labeled. For some, this simply represents a desire to know that the food has been treated in this way so that they are not misled. For others, the demand will clearly enable them to avoid these foods. For still others, it may be that they will see benefits in irradiation for which they are prepared to pay a premium price. And for some, there will be a need to know so that they do not, in fact, have too

REFERENCES

1. *Nutrition and Your Health: Dietary Guidelines for Americans.* US Department of Agriculture and US Department of Health and Human Services, 1985.
2. National Advisory Committee on Nutrition Education, Health Education Council 1983; COMA Diet and Cardiovascular Disease. London: HMSO, 1984.
3. R. W. Smithells, et al. Further Experience of Vitamin Supplementation for Prevention of Neural Tube Defect Recurrences. *Lancet,* 1027-1031, 1983.
4. M. H. Soltan and D. M. Jenkins. Maternal and Foetal Plasma Zinc Concentration and Foetal Abnormality. *British Journal of Obstetrics and Gynaecology,* 89, 56-58, 1982.
5. R. W. Wenlock, et al. *The Diets of British Schoolchildren.* DHSS. London: HMSO, 1986.
6. Hearing of the US Senate Select Committee on Hunger, Washington, DC, April 4, 1986, and Michael F. Jacobson. *The Complete Eater's Digest and Nutritional Scoreboard.* Garden City, NY: Anchor/Doubleday, 1986.
7. Sir Arnold Burgen. Statement to DHSS Press Conference, London, April 10, 1986.
8. Titlebaum, Dubin, and Doyle. Will Consumers Accept Irradiated Foods. *Journal of Food Safety,* 5, 219-228, 1983, and Bruhn, Schultz, and Sommer. Attitude Change Toward Food Irradiation Among Conventional and Alternative Consumers. Vol. 40, *Food Technology,* 86, January 1986.
9. Survey conducted by the UK Consumers' Association, London, November 1986.
10. *Food Irradiation.* Omnibus Research by Marplan Ltd. Conducted for the London Food Commission, London, January 1987.
11. Brand Group. *Irradiated Seafood Products: A Position Paper for the Seafood Industry.* Prepared for the National Marine Fisheries Service, Washington, DC, January 1986.
12. Sanford Miller, FDA Center for Food Safety and Applied Administration, Statement at FDA Consumer Forum, Washington, DC, April 11, 1984.
13. Edward S. Josephson and Martin S. Peterson, eds. *Preservation of Food by Ionizing Radiation* (3 vols.). Florida: CRC Press, Vol. 1 1982, Vols. 2 & 3 1983.
14. P. S. Elias and A. J. Cohen. *Recent Advances in Food Irradiation.* Amsterdam and New York: Elsevier Biomedical Press, 1983.
15. P. S. Elias and A. J. Cohen. *Radiation Chemistry of Major Food Components.* Amsterdam and New York: Elsevier Biomedical Press, 1977.
16. W. Heiman. *Fundamentals of Food Chemistry.* Chichester, England: Ellis Horwood, 1980.

BIOLOGY OF

FOOD IRRADIATION

David R. Murray

RESEARCH STUDIES PRESS LTD.
24 Belvedere Road, Taunton, Somerset, England TA1 1HD

Marketing and Distribution:

Australia and New Zealand:
JACARANDA WILEY LTD.
GPO Box 859, Brisbane, Queensland 4001, Australia

Canada:
JOHN WILEY & SONS CANADA LIMITED
22 Worcester Road, Rexdale, Ontario, Canada

Europe, Africa, Middle East and Japan:
JOHN WILEY & SONS LIMITED
Baffins Lane, Chichester, West Sussex, England

North and South America:
JOHN WILEY & SONS INC.
605 Third Avenue, New York, NY 10158, USA

South East Asia:
JOHN WILEY & SONS (SEA) PTE LTD
37 Jalan Pemimpin #05-04
Block B Union Industrial Building, Singapore 2057

Library of Congress Cataloging in Publication Data

Murray, David R. (David Ronald)
Biology of food irradiation / David R. Murray.
p. cm. — (Research studies in botany and related applied fields ; 5)
Includes bibliographical references.
ISBN 0-471-92621-3 (Wiley)
1. Irradiated foods—Health aspects. 2. Radiation preservation of food. I. Title. II. Series.
RA1258.M87 1990
664'.0288—dc20
89-24087
CIP

British Library Cataloguing in Publication Data

Murray, David R. (David Ronald). *1943–*
Biology of food irradiation.
1. Food. Irradiation
I. Title II. Series
664'.0288

ISBN 0 86380 096 3
ISBN 0 471 92621 3 Wiley

ISBN 0 86380 096 3 (Research Studies Press Ltd.)
ISBN 0 471 92621 3 (John Wiley & Sons Inc.)

Printed in Great Britain by SRP Ltd., Exeter

3.3 Texture

From the time we begin to eat solid food, we develop expectations of texture or 'mouth-feel' of various foods, mediated by the tongue. Food is regarded as unpalatable if it does not conform to our expectations. To a certain degree, assessment of textural palatability depends on cultural background.

Many fruits soften excessively following irradiation, as measured by their decline in physical resistance to impact (Section 2.3.3). However, it may take some days for the consequential changes in softness to affect palatability. Differences in the texture of Granny Smith apples irradiated at 250 Gray, 500 Gray or 1 kiloGray are not noticeable immediately (Eric et al., 1970). After 2 days, only control fruits (unirradiated) retained a characteristic firm crisp texture. After 7 days, the irradiated apples were assessed as soft, pulpy and mushy, whereas the unirradiated apples maintained their condition.

In contrast to apples, irradiated pears do not soften sufficiently, even after prolonged treatment with ethylene, and their texture is judged to be "dry and mealy" (Maxie et al., 1966b). Clearly there is no commercial future for apples that are not crisp, and pears that are not succulent.

In starchy foods like rice, irradiation damages the starch granules to such an extent that rice grains collapse into mush during cooking (Section 4.3.2). The texture of cooked irradiated rice grains is unacceptable to those accustomed to eating rice every day.

CHAPTER 4

Radiolytic Products and Selective Destruction of Nutrients

4.1 Radiolytic Products

An X-ray quantum that makes a random direct hit on the outermost orbital of a molecule can expel an electron that might eventually cause the dislodgement of 2,000 more electrons (Alexander, 1957). This calculation was carried out for irradiated water, but similar principles apply to gamma radiation, and for foods, which are invariably hydrated to some extent. Hydrated electrons, electrically neutral hydroxyl radicals ($OH^{\cdot}$), and negatively charged superoxide radicals ($O_2^{-\cdot}$) are all considered to be the principal early products of irradiation in foods.

The superoxide radical is produced by the combination of a hydrated electron with molecular oxygen, and possibly other oxygen-rich atomic groupings. This molecule is identical with a naturally produced metabolite. However, in living cells the production of superoxide is mainly enclosed in specific subcellular compartments or organelles, each type equipped with at least one form of the enzyme superoxide dismutase (Fridovich, 1983):

$$O_2^{-\cdot} + O_2^{-\cdot} + 2H^+ \longrightarrow H_2O_2 + O_2$$

The hydrogen peroxide produced by this reaction might then be a substrate for the action of peroxidase (p.61) or catalase:

pages 72-78 omitted

4.2 Destruction of Essential Nutrients

Disproportionate and selective losses of essential nutrients occur in foods as a consequence of irradiation. This is because free radicals generated by irradiation do not simply engage in chemical reactions with one another. They behave as selective reagents. It is immaterial that stable radiolytic products are measured in minute concentrations. The essential nutrients that are being irreversibly modified by reaction with free radicals are often present only in low to minute concentrations. It is the proportion of a given nutrient remaining in a food at the time of eating that really matters.

An insidious characteristic of irradiation-induced loss is the way it continues during storage. This has been observed for unsaturated fatty acids, the sulphur-amino acid cysteine, vitamin E, vitamin B_1 and vitamin C as described below. According to the food and storage time, nutritional erosion could be virtually complete in irradiated foods even before taking into account any accelerated losses during cooking (Section 4.4).

4.2.1 Unsaturated Fatty Acids

The double bonds between certain carbon atoms in long chain fatty acids esterified with glycerol are selectively attacked by some of the free radicals produced by irradiation, particularly the superoxide and hydroxyl radicals. When one oxygen atom is gained, a cyclic epoxide can form:

These compounds are highly reactive, forming new addition products that will add an oxygen or nitrogen atom to either side of the oxygen bridge (Burgoyne, 1979). Like the epoxide of vinyl chloride, a potent carcinogen (Bruice, 1986), lipid-derived epoxides could interact with strategic nitrogen atoms belonging to the bases of nuclear DNA,

irreversibly switching on oncogenes that control whether cell division occurs or not.

When two linked oxygens are gained at an unsaturated carbon-carbon bond, they can remain linked to one of the carbon atoms, forming a lipid peroxyl radical. This can in turn react with another carbon-carbon double bond to become a lipid hydroperoxide, thus setting up a chain reaction (Fig. 11). Similar chain reactions are involved in the setting of polyacrylamide gels (Tanaka, 1981).

Unless the ensuing series of reactions is interrupted by an anti-oxidant (Section 4.2.2), superoxide radicals can each facilitate the formation of dozens, hundreds, or even thousands (Gey et al., 1987) of lipid fatty acyl hydroperoxides, which rearrange to form malondialdehyde and other products. The major identified stable products from the irradiation of beef and other meats include alkanes and alkenes, clearly derived from fatty acids (Urbain, 1986; Guldborg, 1986).

E. D. Wills (1980a,b) measured the irradiation-induced formation of lipid hydroperoxides by an iodometric method, and the production of dialdehydes with thiobarbituric acid (Wills and Rotblat, 1964). He chose three different lipid sources, differing in overall fatty acid composition, and mixed each with starch in a ratio 1:9 (w/w). The herring oil contained an appreciable proportion of polyunsaturated fatty acids with more than three double bonds each, the maize oil contained mainly linoleic acid, and the lard contained the lowest amount of polyunsaturated fatty acids but more than 40% mono-unsaturated oleic acid (Table 12). On irradiation, the extent of lipid peroxidation was much higher for the herring oil mixtures than for the other two lipid types, which gave similar results (Table 13).

A number of important findings emerge from Wills' observations. First, electron beams are not as effective as gamma irradiation in generating products of lipid peroxid-

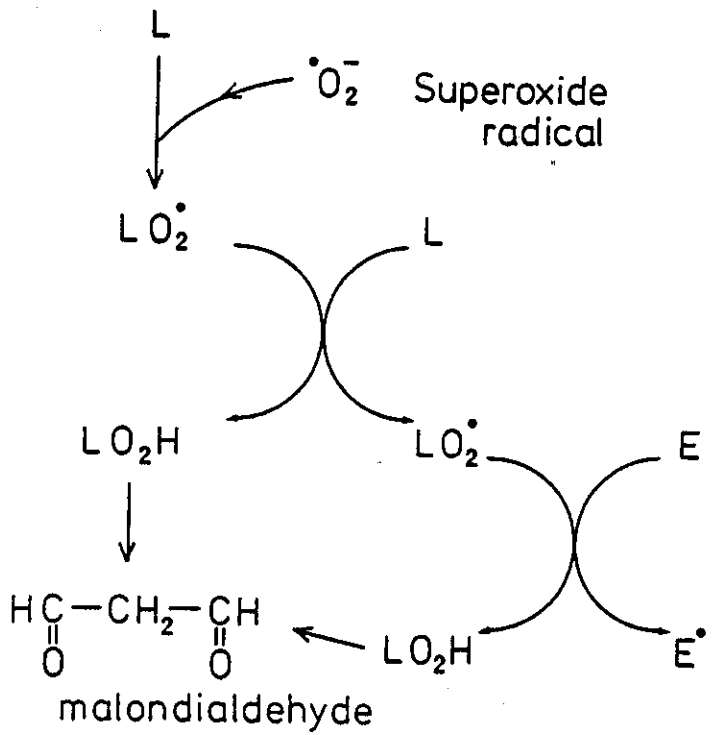


Fig. 11. Superoxide-initiated lipid peroxide formation allows a chain reaction to incorporate oxygen to form successive lipid peroxides. Breakdown products include malondialdehyde, a potent cross-linking agent. Alternatively, a lipid peroxyl radical could be intercepted by a lipid-soluble anti-oxidant such as vitamin E. Key: L, lipid fatty acyl moiety with at least one double bond; $LO_2^\cdot$, lipid peroxyl radical; LO_2H, lipid hydroperoxide; E and E', vitamin E and chromanoxyl radical respectively.

* * * * * *

ation. This reflects lower penetration. Secondly, lipid peroxide formation and breakdown continue in the storage

Table 12.
The Fatty Acid Composition of the Three Lipid Sources Used by E. D. Wills (1980a,b) for Irradiation Studies.

	% of total fatty acids		
Fatty acid[a]	Lard	Maize oil	Herring oil
14:0 myristic	1.8	-	6.6
16:0 palmitic	27.4	13.7	14.4
16:1 palmitoleic	3.4	-	7.2
18:0 stearic	18.3	2.1	2.5
18:1 oleic	41.9	28.7	18.3
18:2 linoleic	7.4	55.2	1.9
18:3 linolenic	-	9.3	1.3
18:4 octadecatetraenoic	-	-	2.9
20:1 eicosenoic	-	-	9.8
20:5 eicosapentaenoic	-	-	16.3
22:1 docosenoic	-	-	7.9
22:5 docosapentaenoic	-	-	0.7
22:6 docosahexaenoic	-	-	10.1

[a]The number of carbon atoms is given first, then after the colon, the number of double bonds. The *cis* configuration is adopted around each double bond. A complete description would also indicate the positions of the double bonds, numbering from the carboxyl end.

* * * * * *

period following irradiation. The rates of peroxide formation early in this storage period depend on the dose, the dose rate, and the temperature ($37^\circ > 21^\circ > 4^\circ C$).

The water content of the starch used in these experimental mixtures affected the rate of lipid peroxidation following irradiation. For a given dose, the rate of lipid peroxidation was maximal at a starch water content of 25%. The water content of grain that has not been fully dried would be similar to this, although most foods would be

Table 13.
Lipid Peroxidation in Herring Oil, Maize Oil or Lard, Each Mixed with Starch and Irradiated with Cobalt-60.

Lipid source[a]	Malondialdehyde formed (nmoles g^{-1}) Control	2 kGy	9.5 kGy
Herring oil	2,853	7,796	18,957
Maize oil	136	167	315
Lard	128	216	380

[a]10% (w/w) with starch. Products of lipid peroxidation were measured by the thiobarbituric acid method immediately after irradiation. Means of three determinations are shown. Data of E. D. Wills (1980a).

* * * * * *

higher (meats, fruits, vegetables) or lower (mature cereal grains and other seeds).

On the basis of E. D. Wills' research it might be argued that the adverse effects of irradiation on unsaturated fatty acids in foods could be avoided by adding anti-oxidants (Section 4.2.2), excluding oxygen, and operating at a very low temperature. But it would be impossible to remove all the water from foods to be irradiated. Attempts to achieve this by deep-freezing would inflict unacceptable structural damage on meats and fish. There are also valid objections to the use of synthetic anti-oxidants such as BHT (butylated hydroxytoluene or 2,6-di-t-butyl-4-methoxy phenol) to which some people are sensitive (Buist, 1986). Although it is a simple matter to include anti-oxidant in a blend of lipid and starch then generate a homogeneous distribution on mixing, it is impossible to ensure an even distribution of added anti-oxidant in real foods like chicken carcasses or meat cuts, which may comprise 30% fat (Greenfield, 1987; Nixey, 1988).

Even if the use of lipid-soluble anti-oxidants were feasible, this would only divert superoxide and hydroxyl radicals from one type of selective reaction. Enhanced destruction of glycosidic linkages in starch or other polysaccharides (Section 4.3) and of sulphur-amino acids (Section 4.2.6) might well occur instead. Lipid peroxidation is less in irradiated herring oil-starch mixtures when casein or ovalbumin is added (Wills, 1980a), an effect which is consistent with the notion of competition among susceptible sites for reaction with superoxide or hydroxyl radicals.

Two aspects of lipid peroxidation cause most concern. First, the partial or complete removal of unsaturated and polyunsaturated fatty acids from food is detrimental, given that these are essential in the human diet. Without *cis* unsaturated fatty acids in membrane phospholipids, the saturated fatty acids would fit together too closely; the membranes would lose fluidity or flexibility, thus inhibiting transport functions.

There is no doubt that plants have the enzymatic capacity to introduce double bonds into fatty acids during lipid synthesis. The most abundant plant sources for human consumption are reserve triacylglycerols in seeds, and in these, the most abundant fatty acids containing double bonds are oleic, linoleic and linolenic acids (Slack and Browse, 1984; Trelease and Doman, 1984). Irradiation of oilseeds would accelerate rancidity, whether at 6 kiloGray (Heilpern 1987) or at 1 kiloGray, the dosage proposed for insect disinfestation.

Secondly, the toxicological aspects of rancidity of irradiated foods deserve close attention. Our aversion to rancid flavours, which stem from the oxidative release of free fatty acids from phospholipids or triacylglycerols, has a sound physiological basis. Effects of consuming rancid fats or oils include low growth rate, infertility in both males and females, hepatic necrosis, red blood cell fragility, and forms of muscular dystrophy (White et al.,

1959). The toxic agents include lipid hydroperoxides, which have the same effects when injected (see Wills, 1980a), and breakdown products such as malondialdehyde.

Lipid peroxides generated by gamma-irradiation can also bring about the formation of stable carcinogenic and mutagenic quinones by reaction with benzpyrenes, which are polycyclic aromatic hydrocarbons (Gower and Wills, 1984, 1986). The 1,6-, 3,6- and 6,12- quinones were identified as major products following irradiation of benzpyrene in a 10% mackerel oil-starch mixture at 3 kiloGray, whether oxygen was present or not. Benzpyrenes themselves are not carcinogenic until converted to quinones (Levin et al., 1978; Gelboin, 1980). These can readily be absorbed from the intestine and circulate to susceptible organs, then generate benzpyrene semiquinone radicals and other mutagenic species *in situ* (McNeill and Wills, 1985; Gower and Wills, 1986).

Accordingly, the smoking of irradiated fish to conceal the off flavour (Section 3.2.2) will enhance the availability of preformed carcinogens from such fish when eaten and for this reason alone should not be permitted.

4.2.2 Lipid-Soluble Vitamins: Vitamin E

Of the vitamins soluble in oils or fats, two in particular are derived from dietary sources - vitamins E and A. Irradiation-induced depletion of these two vitamins in foods that are important sources will be considered in detail. However, two lipid-soluble vitamins, D and K, are not a major concern in the context of irradiation of human foods because we are not totally reliant on dietary sources. Brief exposure of the skin to the ultraviolet component of sunlight is sufficient to ensure adequate synthesis of vitamin D. Deficiencies can appear during winter in parts of the Northern Hemisphere, or in response to covering the skin almost totally in other climates. The traditional

remedy has been cod-liver oil. Vitamin K (menadione and related compounds) is normally supplied by our intestinal flora. No vitamin K deficiency should occur as long as these bacteria remain abundant, and the intestinal uptake of lipids is unimpaired (White et al., 1959; Pike and Brown, 1967).

α-Tocopherol (or 5,7,8-trimethyl tocol) is the most abundant of seven similar compounds with vitamin E activity, differing primarily in the number and positions of methyl groups. These are substituents on the same chromane ring structure that bears the functional hydroxyl group at carbon number six. Projecting from the opposite side of the chromane ring is a trimethyl-tridecyl 'tail', which is fully saturated and facilitates the entry of α-tocopherol into the hydrophobic lipid domain of cell membranes.

Vitamin E is often not listed in tables of food composition, and some uninformed remarks have gained currency to the effect that this is a vitamin in search of a deficiency disease. This is a dangerous misconception. The reasons that vitamin E values are not often recorded are the discrepancies between chemical determination and bioassay (Pike and Brown, 1967), and its abundance in plant foods, eggs and fish. It is a component of chloroplasts in green leaves (Dodge, 1977) and is concentrated in the oils from the embryos of cereal grains and oilseeds. According to Mervyn (1986), seed oils contain the following vitamin E contents (mg per 100 g): wheatgerm 190, soybean 87, maize 66, safflower 49, sunflower 27 and peanut 22. Anyone eating a balanced diet with a variety of plant foods should acquire sufficient vitamin E.

The name 'tocopherol' was coined in 1936, and is based on the Greek word *tokos* for childbirth, signifying the capacity of vitamin E to restore fertility to both male and female rats suffering deficiency imposed by the feeding of rancid fat (White et al., 1959). The main if not

the sole function of vitamin E is that of anti-oxidant, with the ability to interrupt a chain reaction of lipid fatty acyl peroxidation (Fig. 11) and protect membrane structure. This function is well established for plants (Dodge, 1977) and mammals (Pike and Brown, 1967; Menzel et al., 1972; Niki, 1987). Increasing attention has recently been paid to this role in studies on cataract formation in the lens of the eye (Verma, 1987) and in the incidence of heart disease (Gey et al., 1987). The latter study suggests that a blood plasma concentration less than 0.9 mg α-tocopherol per 100 mL, in conjunction with poor vitamin C status, indicates a high risk for ischaemic heart disease.

Superoxide radicals generated by irradiation can potentially interact with the double bonds of fatty acids esterified in the phospholipids of cell membranes, leading to the formation of lipid peroxyl radicals and their breakdown products (Section 4.2.1). By 'scavenging' such a radical (Fig. 11), α-tocopherol prevents the lipid moiety from engaging in further generation of lipid free radicals at the expense of carbon-carbon double bonds. Vitamin E is itself oxidized to the chromanoxyl free radical, which can be reduced again to α-tocopherol by ascorbate (Fig. 12). However, if there is insufficient vitamin E to contain the chain reactions of lipid peroxidation, then both vitamin E and vitamin C will be exhausted.

When rolled oats were irradiated at 1 kiloGray, 85% of the original α-tocopherol content had disappeared by the end of 8 months storage (data of J. F. Diehl (1979), as cited in Raica et al. [1972]). This loss occurred in two stages: 20% immediately following irradiation, and a further 65% during storage. The combined loss was more than 3-fold greater than the loss normally entailed in storage. This was 26% for unirradiated oats stored under the same conditions.

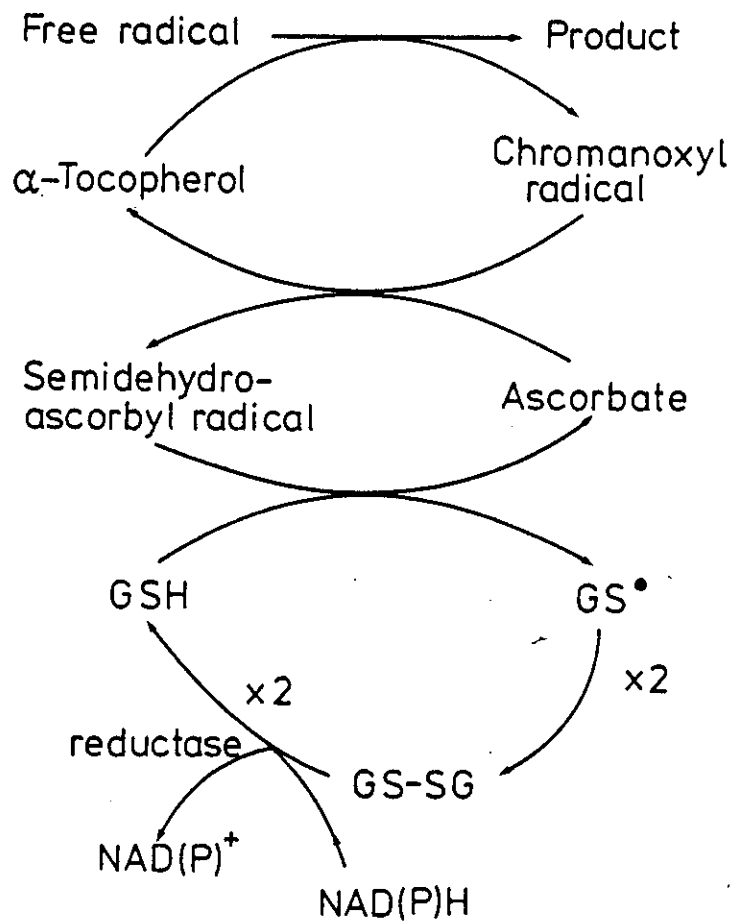


Fig. 12. Redox reactions in the cytosol linked to the regeneration of vitamin E *in vivo*. GSH represents the reduced form of glutathione, GS-SG is the oxidized form, and GS• is the glutathiyl radical. The formulae for ascorbate and semidehydroascorbyl radical are shown in Figure 13.

Vitamin E is more sensitive to immediate radiation damage than a range of synthetic anti-oxidants tested in herring oil-starch mixtures (Wills, 1980b). This destruction is concentration dependent; all anti-oxidants tested were partially or completely destroyed by doses of 1 kiloGray or more, unless they were supplemented to bring their concentration up to 0.1% (w/w), which is sufficient to protect lipids against peroxidation resulting from radiation-generated free radicals in this experimental system (E. D. Wills, 1980a,b).

4.2.3 Lipid-Soluble Vitamins: Vitamin A and Carotenoids

The best defined function of vitamin A (all-*trans*-retinol) is as a constituent of the visual pigment rhodopsin in the retina of the eye. Reserves of retinyl esters in the liver provide a buffer against fluctuating supplies of retinol itself, or carotenoid precursors in the diet. Retinol consists of a six-membered carbon ring built of four isoprene units, terminating with an alcohol group. The alternation of single and double bonds (conjugation) throughout the side-chain is one of the invariant features conferring full vitamin A activity. Using bioassays, Ames (1965) established that no significant feature of the carbon skeleton could be varied without marked or total loss of biological activity. Retinyl esters (e.g. retinyl acetate or retinyl palmitate) remain 100% active, but most modifications of the terminal alcohol group, including oxidation to a carboxyl group, destroy vitamin A activity.

In view of the high degree of unsaturation in retinol, it is to be expected that superoxide or hydroxyl radicals generated by irradiation would interact with these bonds. Consistent with this expectation, dairy foods, collectively one of the richest natural sources of retinol, are substantially depleted of their retinol content by irradiation. According to the summary prepared by Webb and Lang (1987), these losses extend to 47% in cheese, 78% in butter, and 70% in milk.

The situation is not so clear-cut for foods enriched in carotenoid pigments, only some of which serve to any useful extent as precursors of vitamin A. Retinol can be made from half a suitable carotenoid precursor once its central double bond is broken. This primary cleavage is now considered to take place in cells of the intestinal mucosa, rather than in the liver (Pike and Brown, 1967). But uptake and conversion processes are not 100% efficient, and even the best precursor, all-*trans*-β-carotene, is not considered to yield more than 50% retinol. The arbitrary International Unit of Vitamin A is set at 0.3 µg retinol, or 0.344 µg retinyl acetate, or 0.6 µg β-carotene (Ames, 1965).

There is evidence that β-carotene itself serves essential and protective functions in cell membranes (Section 6.5.4), and the inefficiencies in its uptake and conversion to retinol ensure that some β-carotene is retained as such. A few other carotenoids yield retinol with efficiencies estimated between 30 and 50%, but most of the more than 400 known carotenoid pigments show zero pro-vitamin A activity in humans (Bauernfeind, 1981).

Many nutritional analyses for fruits and vegetables have not distinguished β-carotenes from total carotenoids. An attempt is usually made to guess the 'β-carotene equivalent' of retinol by dividing the total carotenoid value by a factor of 6, following the FAO/WHO Expert Group's Report (1967). This factor is based on the 50% conversion factor mentioned above, combined with the assumption that "the availability of β-carotene be taken as one-third". It was not intended that the factor of one-sixth should be applied to total carotenoids, nor is it sufficiently accurate for β-carotenes.

Even among the pigments classed as β-carotenes, some have much lower vitamin A potential than all-*trans*-β-car-

otene (Gebhardt et al., 1977). Consequently, more detailed chromatographic separations and analyses are required in order that a realistic estimate of the pro-vitamin A potential of plant carotenoids can be made. Adopting such procedures for a range of stone fruits, Wills et al. (1983) showed that revised values for pro-vitamin A potential were up to 4-fold lower than those previously published in tables of food composition.

Irradiation of fruits and vegetables can interfere with the normal synthesis of carotenoid pigments (Sections 2.3.3 and 3.1.1). Even when irradiated fruits look as though they might have a normal complement of pigments, no detailed analyses of the kind recommended by Gebhardt et al. (1977) have been undertaken to determine how the pigment composition has altered as a consequence of irradiation. Apart from changes in the pattern of synthesis, carotenoids present at the time of irradiation could readily be modified at a single carbon-carbon bond, and so become devoid of vitamin A potential (e.g. Tinsley et al., 1970). Fruits would not need to be 'bleached' to the same extent as irradiated oranges for this to happen.

At present, values reported for 'β-carotene equivalent' of irradiated fruits are just as meaningless as they are for unirradiated fruits. This kind of approximation conceals selective changes that could indicate that irradiation diminishes the pro-vitamin A potential of carotenoids retained following irradiation.

In situations where people are already malnourished, the β-carotene content of the food becomes irrelevant, because carotenoids can no longer be taken up by the intestinal mucosa, and retinyl esters stored in the liver cannot be mobilized (Scrimshaw and Young, 1976). The immediate remedy for vitamin A deficiency is an injection of retinyl esters.

4.2.4 B-group Vitamins

The B-group vitamins are as distinct from one another biochemically as from any other vitamin (Table 14). Historically, this complex was originally treated as a single water-soluble factor, expanded into a dozen factors, then consolidated so that only B_1, B_2, B_3, B_6 and B_{12} consistently retain the B-group nomenclature.

Table 14.

Major Functions of the B-group Vitamins and Approximate Daily Requirements for Adults.

Vitamin	Daily adult requirement	Example of cofactor function
Thiamin (B_1)	1.0-1.5 mg	Thiamin pyrophosphate aids C_2-group transfer between sugar phosphates.
Riboflavin (B_2)	120 µg per 1,000 kilojoules	Succinic dehydrogenase in the Krebs cycle; desaturation of fatty acids.
Niacin or nicotinic acid (B_3)	up to 18 mg according to tryptophan intake	Precursor of nicotinamide adenine dinucleotide, NAD^+ and $NADP^+$ (reduced forms: NADH, NADPH).
Pyridoxine, pyridoxamine, pyridoxal (B_6)	1.4 mg	Transfer of amino groups in synthesis or breakdown of amino acids.
Cobalamin (B_{12})	2-3 µg	Conversion of ribonucleotides to deoxy- forms for DNA synthesis[a].

[a] Babior (1975).

* * * * * *

All mammals need a dietary supply of riboflavin, pyridoxine and cobalamin. Only specialized herbivores can do without dietary thiamin, relying instead on gut flora.

Niacin, however, can be synthesized from the essential amino acid tryptophan. All niacin taken in from food lowers the amount that has to be formed from tryptophan, thus reducing the likelihood that tryptophan will become limiting in the sense that there might be insufficient to support the demands of protein synthesis (Section 4.2.6). For people with marginal or unbalanced protein intake, a superimposed reduction in niacin intake as would occur with irradiated foods would only compound the problem of malnutrition, rather than alleviate it.

Thiamin is a water-soluble heterocyclic compound, comprising a pyrimidine ring linked to a thiazole ring, which contains atoms of carbon, nitrogen and sulphur. The thiazole ring is particularly susceptible to oxidation, forming a bridge with the NH_2- group projecting from the pyrimidine ring to become thiochrome. This product exhibits blue fluorescence, which provides a convenient assay.

The richest natural sources of thiamin include developing seeds of legumes such as the garden pea (see Table 19) and mature seeds of nuts like the almond (Thomas and Corden, 1970). A yeast extract such as 'Vegemite', although a concentrated source, is consumed in only minute amounts - a spread of 0.1 g would be needed to yield 10 µg. Moderate sources include meats, fruits, vegetables and cereals. A high proportion of the original grain content of thiamin can be lost during physical processing, indeed the discovery of thiamin came from observations that the husks of rice, removed in polishing, were enriched in a factor that prevented beri-beri (White et al., 1959). Even after baking, bread retains about 200 µg per 100 g, equivalent to four slices, and is therefore a useful source depending on how much is eaten.

Wheat flour that was irradiated at 250 Gray lost 67% of its original thiamin content after storage for 8 months (data of J. F. Diehl [1979] cited in Raica et al. [1972]).

There were two components of this irradiation-induced loss - a 20% loss measured soon after irradiation, and a further 47% loss during storage. By comparison, a reduction of only 25% in the original thiamin content was observed during 8 months storage of unirradiated wheat flour.

Accelerated erosion of nutritional value is just one detrimental result of irradiating wheat grain or flour. Other impediments to recommending irradiation as an insecticidal measure are the existence of safer and more effective alternative treatments (Section 2.1), a substantial loss of baking quality, and selective survival of spores of *Aspergillus*, which under certain conditions could lead to unsuspected aflatoxin production (Chapter 5).

Of all the B-group vitamins, thiamin often appears to be the most susceptible to irradiation-induced modification and loss, in meats as well as plant foods (Urbain, 1986). However, the other B-group vitamins can also suffer substantial losses, e.g. riboflavin in irradiated milk and dairy products (up to 74% loss), vitamin B_6 in irradiated beef stored for 15 months (91% loss; Urbain, 1978), and even the heat-stable vitamin B_{12} is depleted by 33% in irradiated milk and meat (Webb and Lang, 1987).

4.2.5 Vitamin C

Vitamin C (L-ascorbate) is a γ-lactone synthesized from D-glucose in most organisms, but it is essential in the diet of humans, other primates, the guinea pig, and some frugivorous mammals such as bats (Howe, 1986). A daily intake of 10-15 mg is sufficient for us to avoid scurvy, but inadequate to support the full range of protective or regulatory reactions that ascorbate undertakes (Table 15). These reactions take place in organs and tissues that are able to acquire and maintain high concentrations of ascorbate, such as the pituitary gland, the adrenal medulla, the liver, the cornea and lens of the eye, white blood

Table 15.

Some of the Metabolic Functions Established for Ascorbate.

Functions	References
<u>As a powerful reducing agent</u>:	
- protection of membrane lipids from peroxidation, directly or via recovery of vitamin E	Varma (1987) Niki (1987) Figure 12.
- provision of proton gradients in membranes, e.g. of Golgi vesicles	Morré et al. (1987).
- participation in hepatic mixed function oxygenase (MFO) and flavin mono-oxygenase (FMO) systems linked to cytochrome P-450	Zannoni et al. (1987) Bruice (1986) Groves (1986).
<u>As a hydroxylation cofactor</u>:	
- proline hydroxylase, necessary for collagen synthesis	Stone and Meister (1962).
- cholesterol 7-α-hydroxylase (bile acid synthesis in liver)	Ginter and Jurcovicová (1987).
- 4-hydroxyphenylpyruvate dioxygenase and dopamine β-mono-oxygenase (two steps in catecholamine synthesis in adrenal medulla)	Diliberto et al. (1987) Levine and Hartzell (1987).
- five other enzyme activities	" " " " ".
<u>As a nitrite scavenger</u>:	
- stoichiometric reaction of nitrite with ascorbate blocks formation of nitrosating agents, which would otherwise form mutagenic and carcinogenic compounds, such as nitrosamine	Tannenbaum and Wishnok (1987).

cells, seminal vesicles and released sperm.

Dehydroascorbate is the full oxidation product of ascorbate (Fig. 13). Although this compound could notionally be

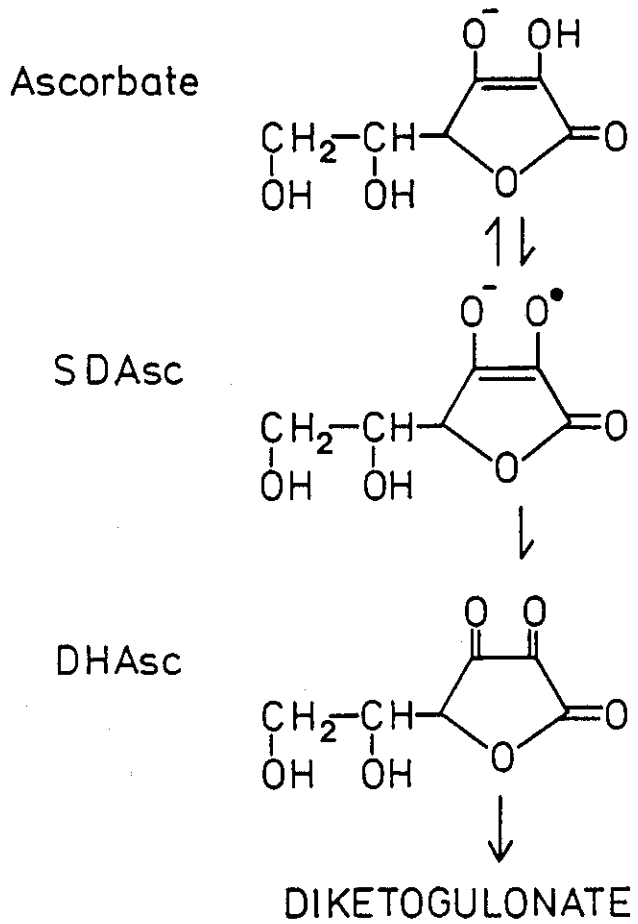


Fig. 13. Redox reactions of ascorbate, semidehydroascorbate (SDAsc) and dehydroascorbate (DHAsc).

* * * * * *

reconverted to ascorbate by an input of reducing potential this expectation is not fulfilled *in vivo*. Ascorbate is often consumed irreversibly as an anti-oxidant (Chatham et al., 1987), and derivatives of dehydroascorbate such as diketogulonate, methyl glyoxal and oxalate are not recov-

erable through further metabolism (Lohmann, 1987a,b).

Daily intakes of ascorbate in the range 45-100 mg are desirable, and probably optimal under most circumstances (Kallner, 1987). These amounts are greater than the 30 mg recommended for adults by the WHO Joint Expert Group (WHO, 1970), a figure which is still accepted uncritically by dieticians and physicians. The amount needed can vary from day to day. Exposure of the lungs to air pollutants increases the requirement for ascorbate in the lungs. This has been demonstrated with ozone (Kratzing and Willis, 1980) and tobacco smoke. Exposure of the lungs to tobacco smoke, with its broad array of xenobiotics, increases the expenditure of vitamin C to the extent that regular smokers would need to ingest about 60 mg ascorbate per day more than non-smokers in order to maintain similar concentrations of ascorbate in the blood serum (Smith and Hodges 1987).

Serum concentrations of ascorbate respond rapidly to dietary increase or decrease (Jacob et al., 1987) and over a threshold of about 0.3 mg per 100mL are a good indicator of whole body vitamin C status (Omaye et al., 1987). There is a strong correlation between deficiency and increased risk of death from ischaemic heart disease in certain populations in Finland and the British Isles (Gey et al., 1987).

The major dietary sources of vitamin C are fruits and vegetables, except for people like the eskimos, who rely on the organs of freshly killed animals. The most concentrated plant sources include capsicum, guava, papaya, citrus fruits, strawberries, cauliflower, cabbage, broccoli, kale and watercress, which generally contain at least 50 mg ascorbate per 100 g edible portion. Even unripe green capsicums have more than this, and according to cultivar, ripe red capsicums can attain up to 300 mg per 100 g (Khadi et al., 1987). Less concentrated but nonetheless

important sources, as judged by actual consumption, include pineapple, stone fruits, tomato, peas, beans, sweet potato, potato and onion (see Tables 16, 19, 20, 21).

Many studies have shown that irradiating fruits and vegetables causes a substantial or total depletion of their vitamin C content (Maxie and Sommer, 1968; ACINF, 1986). Other studies purport to show little or no loss of vitamin C due to irradiation. Apart from the kind of fruit or vegetable and the dosage, an important variable to be considered is the length of time elapsed following irradiation before the analysis is performed, as in lemons (Fig. 14). Clearly the losses 5 days after irradiation are nowhere near as severe as the losses 40 days afterwards. Since the extension of storage life is one of the most frequently stated aims of proponents of irradiation, the losses in vitamin C content accrued following irradiation and storage are of far greater import than the marginal effects apparent within the first few days.

Irreversible loss of at least some vitamin C is always to be expected following irradiation. Even in simple experimental solutions, ascorbate reacts with free radicals generated by gamma-irradiation. An irreversible and total loss of ascorbate cannot be prevented by high concentrations of thiol reagents (glutathione or cysteine) present in the irradiated solutions (Rose, 1987).

A Joint Expert Committee on Food Irradiation (JECFI, 1981) suggested that irradiation of plant foods converts some of their ascorbate content to dehydroascorbate, which is then not measured by the conventional dye reduction assay. Dehydroascorbate and ascorbate were considered by this Committee and by ACINF (1986) to have equivalent biological activity. Thus if dehydroascorbate were to be assayed and its content added to the ascorbate value, the effective loss of vitamin C as a consequence of irradiation might be much less than assays of ascorbate alone have

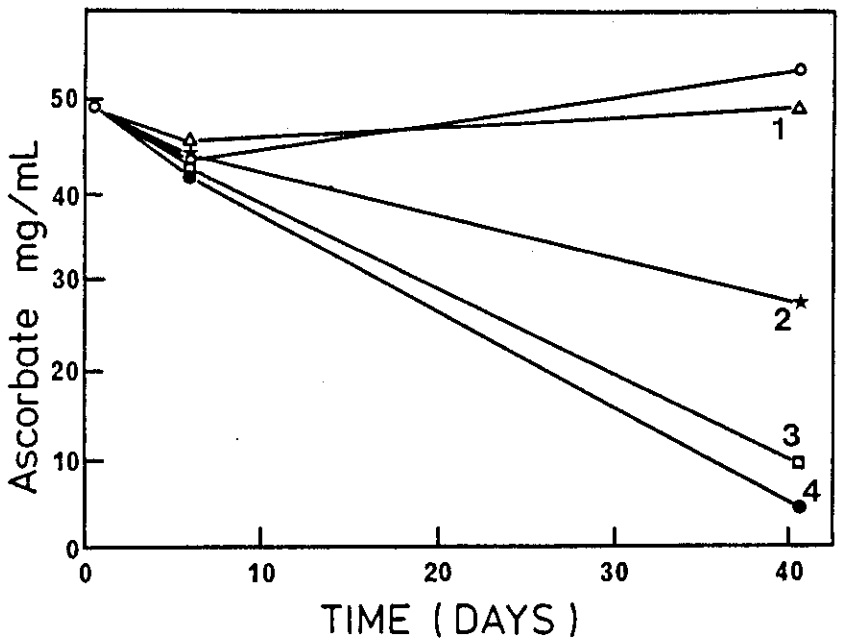


Fig. 14. Effects of gamma radiation applied on the second day on the ascorbate content of Eureka lemons maintained at 15°C. The dosage was 1, 2, 3, or 4 kiloGray as indicated. Ascorbate values are mg per mL of expressed juice. Data of Maxie, Eaks and Sommer (1964a).

* * * * * *

indicated (JECFI, 1981; Cumming, 1988).

But ascorbate and dehydroascorbate do not have equal biological activity. Dehydroascorbate is an oxidation product of ascorbate, derived in two steps, each involving the transfer of a single electron (Fig. 13). It cannot be utilized instead of semidehydroascorbate (ascorbyl radical) in reversible enzyme-catalysed redox reactions (Table 15). Dehydroascorbate is more likely to be formed in the non-enzymic chemical reactions which ascorbate undergoes;

Table 16.

Vitamin C Contents of Fruits and Vegetables According to Different Assay Procedures (mg per 100 g edible portion).

Food	Ascorbate only, by titration with dye[a]	Ascorbate plus dehydroascorbate, fluorimetric
Beans (green)	21	21[b]
Peas (shelled)	26	32[b]
Apricot	8	7 or 16 (two cv.)[c]
Nectarine	19	11 or 13 (two cv.)[c]
Peach	7	6 to 16 (8 cv.)[c]
Plum	4	4 to 6 (6 cv.)[c]
Cherry	8	16 to 21 (3 cv.)[c]
Pineapple	26	13[d]
Pear	4	5[d]
Tomato	22	16.5[d]

[a]Thomas and Corden (1970) [b]Wills et al. (1983)
[c]Wills et al. (1984) [d]AGAL (1988).

* * * * * *

it is unstable and spontaneously forms diketogulonic acid, which is "devoid of antiscorbutic or other metabolic activity" (Rose, 1987).

In routine analyses of fruits and vegetables, ascorbate alone should be considered as vitamin C. Estimates based on summation of ascorbate and dehydroascorbate contents determined by microfluorimetry or HPLC (high performance liquid chromatography) are, however, little different in many instances from estimates based on reduction of the dye 2,6-dichlorphenolindophenol (Table 16). The appreciable differences that have been observed go in either direction (e.g. cherries and pineapple) and can be attributed to cultivar or growth conditions rather than to the presence of sizeable quantities of dehydroascorbate. There is no evidence to support the view that a substantial res-

ervoir of dehydroascorbate has been overlooked by analysts in the past.

Irradiation-induced losses of ascorbate in plant foods are real losses. Once such foods are impoverished of their original ascorbate content there is no miraculous way of restoring it. The fall-back argument is that such losses are acceptable after all, because substantial losses of vitamin C already occur on processing by alternative means and cooking. This proposition is discussed in Section 4.4.2.

4.2.6 Amino Acids

Amino acids are ingested mainly as proteins, which consist of one or more types of polypeptide chain. Peptide bonds link neighbouring amino acid residues. During digestion, these bonds are hydrolysed by a range of proteolytic enzymes with distinct specificities (including trypsin, chymotrypsin, aminopeptidases and carboxypeptidases) and the liberated amino acids are taken up and transmitted by the intestinal mucosa.

Approximately half the common protein-forming amino acids are said to be essential in the human diet, because their synthesis may be confined to certain tissues (e.g. arginine) or blocked altogether through the lack of appropriate enzymes. The essential amino acids are indicated in Table 17, which records the complete amino acid analysis for ovalbumin. This is the major reserve protein of egg white (hen eggs), and is one of the conventional reference standards for protein quality.

It is equally necessary to ingest 'non-essential' amino acids and amides in order to avoid metabolic losses of the essential amino acids, one or more of which may be in limiting amount, according to the quality of our dietary protein sources. In a mixed diet with daily input from dairy foods, eggs, meat or fish, none of the essential amino

Table 17.

The Amino Acid Composition of Ovalbumin from Hen Eggs, Compiled from the Data of McReynolds et al. (1978).

Amino acid	Number per polypeptide	% of Total residues
Aspartate	14	3.64
Asparagine	17	4.41
Glutamate	33	8.57
Glutamine	15	3.90
Threonine[a]	15	3.90
Serine	38	9.87
Glycine	19	4.93
Alanine	35	9.09
Valine[a]	31	8.05
Cysteine[b]	6	1.56
Methionine[b]	16	4.16
Isoleucine[a]	25	6.49
Leucine[a]	32	8.31
Tyrosine[c]	10	2.60
Phenylalanine[c]	20	5.19
Tryptophan[a]	3	0.78
Histidine[d]	7	1.82
Arginine[d]	15	3.90
Lysine[a]	20	5.19
Proline	14	3.64

[a]essential *per se* [b]considered jointly, fulfilling requirement for combined sulphur [c]considered jointly, fulfilling pre-formed benzene ring requirement [d]essential in infancy and childhood.

* * * * * *

acids should ever be limiting. But in vegetarian diets with cereal grains or starchy tubers as main protein and energy sources, deficiencies will occur, particularly of lysine, tryptophan, methionine and threonine. Such diets

should whenever possible be complemented with legume seeds or seedlings (Section 2.3.1) and oilseeds. The seed proteins of many cultivated legumes will provide adequate lysine, threonine and tryptophan (Sastry and Murray, 1986) and chick peas provide the best legume seed source of the sulphur-amino acids, methionine and cysteine (Bhatty, 1982; Murray and Roxburgh, 1984; Murray, 1979, 1984a; Sastry and Murray, 1987).

The carbonyl groups of peptide bonds are far more stable to the presence of free radicals following irradiation of foods than are the corresponding carbonyl groups in the ester linkages of acyl glycerols (Section 4.2.1) or the glycosidic linkages between sugar units in polysaccharides (Section 4.3.2). As most peptide bonds remain unbroken following irradiation of foods up to at least 10 kiloGray, it is incorrect to equate the effects of irradiation with "pre-digestion" (Cooper, 1988). However, the side-chains of amino acid residues within polypeptide chains are not necessarily as stable as peptide bonds. Many reviewers have missed the point that irradiation damage to proteins in foods is selective; certain of the amino acid residues are more susceptible to modification than others (Murray, 1988a).

Amino acids with a benzene ring (phenylalanine, tyrosine), or a heterocyclic structure such as the indole ring of tryptophan, are prone to hydroxylation by hydroxyl free radicals. The formation of novel protein-bound isomers of tyrosine following irradiation has already been mentioned (Fig. 7). There is also a coherent body of evidence for selective loss of the essential sulphur-amino acids, methionine and cysteine, through a variety of reactions.

Sulphur-containing compounds are not only prominent among stable irradiation products - they can comprise the most abundant category of such products, as in irradiated

beef (Urbain, 1986). These products include dimethyl sulphide, dimethyl disulphide, ethyl mercaptan and hydrogen sulphide. Hydrogen peroxide is known to convert methionine to methionine sulphoxide (Shrift, 1966). Methionyl residues in polypeptides will react with either hydrogen peroxide generated as a radiolytic product, or superoxide radicals, to yield methionine sulphoxide as one breakdown product.

Dimethyl disulphide (CH_3-S-S-CH_3) might be derived from two CH_3-S$^{\cdot}$ radicals liberated from the terminal region of methionine residues (Fig. 15), or else from the methylene groups of two cysteine residues linked by a disulphide bridge. These bridges are a highly conserved structural feature of correctly folded polypeptide chains, providing stability:

$$|\text{-}CH_2\text{-}SH \quad + \quad |\text{-}CH_2\text{-}SH \longrightarrow |\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}|$$

When proteins are hydrolysed at their peptide bonds, certain pairs of cysteines are always found with the disulphide bridge intact (i.e. as cystine), unless reagents are applied to reduce the bridges to free thiol groups, or to convert all cysteine residues to *S*-sulphocysteine (Inglis and Liu, 1970). In the case of ovalbumin (Table 17), two of the six cysteine residues always form a disulphide bridge, whereas four retain free thiol groups (Betteridge et al., 1974; Thompson and Fisher, 1978).

In addition to their functions within polypeptides, sulphur-amino acids are essential in our diets for the donation of methyl groups, and the synthesis of glutathione, the tripeptide γ-glutamyl-cysteinyl-glycine (Fig. 15). Legume seeds may provide some cysteine as glutathione (Spragg and Yemm, 1954; Murray, 1988b). This tripeptide has a redox function involving the thiol group of its central cysteine residue. The fully oxidized form possesses a disulphide bridge evidently formed from two thiyl radicals

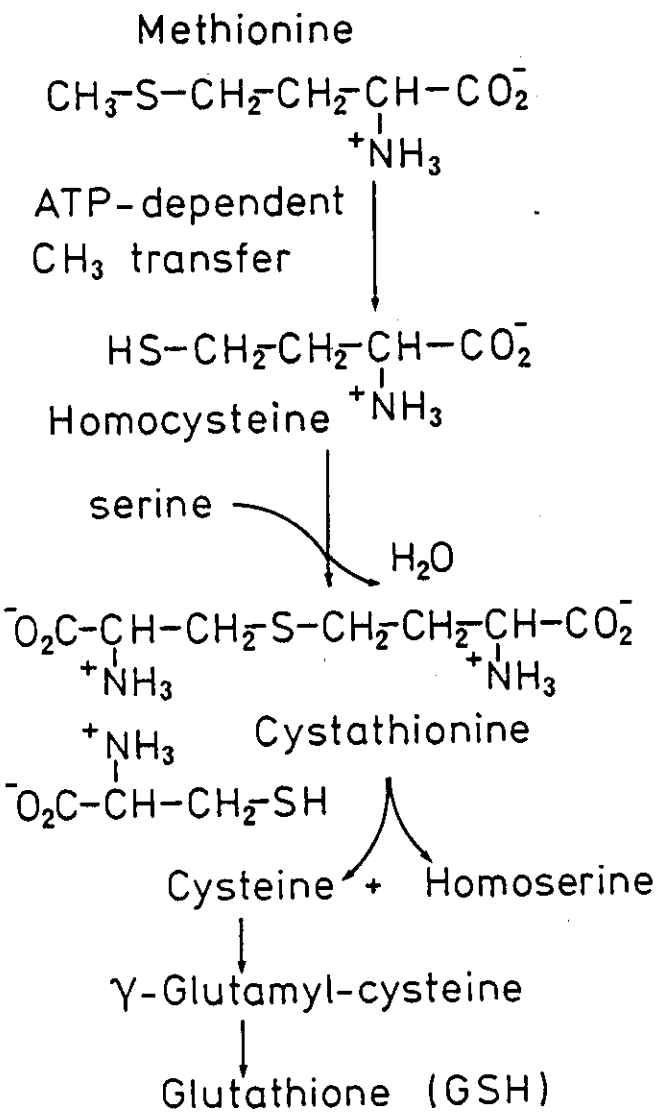


Fig. 15. Metabolic relationships between methionine, cysteine and glutathione in the liver. Cysteine cannot be used to synthesize methionine, but methionine can be spared for protein synthesis by cysteine derived from the diet.

(Niki, 1987; Fig. 12).

There are scattered reports of appreciable losses of methionine from proteins of irradiated foods. The loss of thiol groups, enhanced after 1 month's storage, provides one way of detecting whether a food has been irradiated or not (Guldborg, 1986). Selective loss of cysteine following irradiation is illustrated by the data and calculations in Table 18. Losses of disulphide bridges following irradiation can destabilize or denature some proteins (Urbain, 1978, 1986; Section 5.3).

Table 18.

Effects of Gamma-Radiation on Recovery of Three Essential Amino Acids from Beef (each as g per 100 g protein).

Treatment	Histidine	Tryptophan	Cysteine
Unirradiated	3.43	1.703	1.27
20 kiloGray	3.44	1.477	0.89
Loss due to irradiation:	ND	13.3%	29.9%

From data of J. F. Diehl (1983). ND. not detectable.

* * * * * *

It is noteworthy that the Joint Expert Committee on Food Irradiation (JECFI, 1981) expressed reservations about the irradiation of staple protein sources, and called for further studies on the effects of irradiating legume seeds. The results of such studies, if they eventuate, are broadly predictable. If vegetarians with marginal or already deficient protein intakes are forced to accept irradiated cereal grains and legume seeds, existing amino acid deficiencies will be exacerbated, and marginal sufficiencies will be undermined. Protein malnutrition will become more widespread, especially amongst pre-school children, whose protein requirements are high in relation to energy needs (Bhatia and Rabson, 1987). This consequence is unavoidable because with few exceptions (Table 17), one essential

amino acid cannot substitute for another.

4.3 Modification of Carbohydrates

4.3.1 Organic Acids

Although not generally recognized as essential nutrients, organic acids such as citric and malic acids are abundant in fruits and developing seeds (see Murray, 1988b). They are certainly useful nutrients, being capable of entering the Krebs cycle as energy sources. As carboxylate anions, they chelate cations and assist in charge balance (Kennedy 1986). Their presence and concentrations in fruits are crucial parameters of quality, related to the pH of the expressed juice. The acidity and pH of wine grapes, for example, are monitored closely in the days leading up to harvest and vintage.

There is very little information on the effects of irradiation on the organic acid contents of fruits, but one study of lemons found substantial dose-related losses in citrate content after 40 days storage at 15°C (Fig. 16).

4.3.2 Sugars and Polysaccharides

The immediate softening of fruits following irradiation (Section 2.3.3) cannot be attributed to accelerated enzymic hydrolysis of cell wall polysaccharides (Maxie and Sommer, 1968), but rather to widespread breakage of glycosidic linkages (-O-) between sugar units, specifically due to reactions with free radicals generated by irradiation. Pectins, for example, fragment following irradiation (Skinner and Kertesz, 1960). Similar fragmentation of starch to form smaller dextrins, maltose and glucose, as well as formic acid, formaldehyde, acetaldehyde, glycolaldehyde, methanol, lactones, H_2O_2, and other low molecular weight products has also been documented (Urbain, 1986). Substantial reduction in viscosity is the most serious consequence of irradiating a starchy food, because

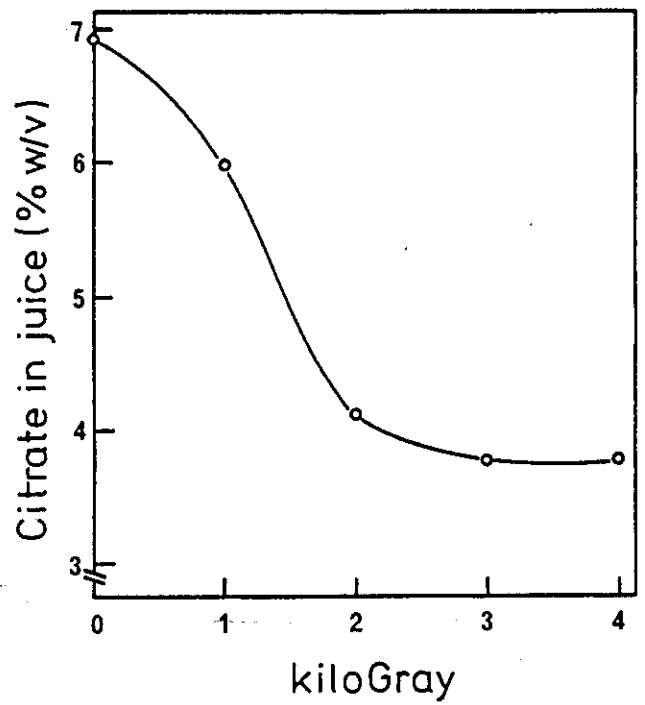


Fig. 16. Effect of gamma-irradiation on the citrate content of juice obtained from Eureka lemons stored for 40 days at 15°C. Data of Maxie, Eaks and Sommer (1964a).

* * * * * *

this affects cooking behaviour (Section 5.3).

Since the radiolytic products from sugars are measured in parts per million, the percentage losses in digestible sugars are minute. The nutritional consequences of irradiation are therefore not serious as far as sugars are concerned. Nevertheless, the adverse structural changes brought about by irradiation have serious consequences for the market prospects of the foods concerned, e.g. fruits and vegetables. These consequences are unavoidable, since even under oxygen-free conditions, hydroxyl radicals will

still be abundant. Protection of polysaccharides afforded by the presence of protein in the same foods (Urbain, 1986) is no consolation, given the selective nature of damage to proteins (Section 4.2.6). Damage to both the polysaccharide and protein constituents of cereal grains like bread wheat destroys baking quality as well as eroding the nutritional value of the food (Section 5.3).

4.4 Synergistic Effects of Irradiation on Nutrient Losses

"I would not get too hung up on the synergistic effect."
- F. J. Cumming (1988)

Synergism is the interaction of two or more causative factors such that their combined effect is greater than the sum of their effects individually. The term is applicable to irradiation because there is evidence that the loss of nutrients from irradiated foods after cooking outweighs the sum of (1) the loss induced by irradiation then storage, and (2) the loss brought about by cooking the same unirradiated food.

If irradiation were to be applied to the meats, fruits and vegetables that form the bulk of most human diets, the nutritional impoverishment of those diets would be so extensive that sufficiency thresholds for many essential nutrients would no longer be met. The phrase 'empty calories' would take on a new dimension.

4.4.1 Irradiation-Induced Loss of Nutrients from Meat

Food Chemical News (1986) quoted the U.S. Agriculture Department's Agriculture Research Service as reporting "a highly significant interaction" between cooking and irradiation of bacon and its content of thiamin (Section 4.2.4). Thiamin was lost at a faster rate during cooking if the bacon had been irradiated: "The two processes, irradiation and cooking, produced degradation, but when the product was cooked after it had been irradiated the overall effect was greater than the sum of the processes applied individually."

There are two components of nutrient loss during the cooking of meat: a chemical lability of vitamins such as thiamin at the higher temperature, and the physical exudation or 'drip loss' of soluble nutrients from the meat as it shrinks during cooking, e.g. on grilling bacon or steak. Drip loss of all soluble nutrients is certain to be exacerbated when irradiated meats are cooked, because the exudation process begins and becomes obvious during storage. Lamb and pork meats irradiated at low temperature after vacuum packaging are known to 'weep' excessively during storage. The slimy appearance of such meats offered for sale would obviously deter customers. Shay et al. (1988) considered this effect a significant loss in quality, to be balanced commercially against any marginal extension in storage 'life'.

4.4.2 Irradiation versus Alternative Processing and Cooking of Plant Foods

The argument that irradiation-induced losses of vitamins from foods can be tolerated because these losses are similar to those sustained on cooking or processing by other methods is specious - it collapses on closer scrutiny. First, the losses due to irradiation must include the extra losses entailed in storage following irradiation, which can severely outweigh the losses normally observed on storage of unirradiated foods (Section 4.2). Secondly, the irradiation-induced losses imposed on fruits would be incurred through a processing step which presently does not exist for these fruits. Thirdly, the actual retention of B-group vitamins and ascorbate after cooking at the temperature of boiling water is often close to 80% or even better (Table 19), depending on the vitamin, the food, and care being taken not to overcook. Frozen vegetables when cooked give similar vitamin retention to fresh vegetables

Table 19.

Retention of Vitamins in Vegetables After Cooking; Amounts are mg per 100 g Edible Portion, and Expressed as a Percentage Compared to the Same Material Uncooked.

Vegetable	Thiamin mg	Thiamin %	Niacin mg	Niacin %	Ascorbate mg	Ascorbate %
Broccoli[a]	0.09	90	1.0	91	92	79
Brussels sprouts[a]	0.08	84	0.6	86	83	86
Carrot[a]	0.05	82	0.5	83	4	67
Peas (green)[a]	0.25	78	2.0	77	20	77
Peas (green)[b,c]	0.23	74	1.4	61	14	44[d]
Beans (pods)[a]	0.06	77	0.4	80	12	57
Beans (pods)[b]	0.03	75	0.3	75	13	62[d]
Potato[a]	0.10	94	1.2	92	11	79

[a]Thomas and Corden (1970) [b]Wills et al. (1984)
[c]submerged in water; steaming is preferable
[d]ascorbate plus dehydroascorbate (Section 4.2.5).

* * * * * *

(e.g. Wills et al., 1984).

Such good retention of ascorbate after cooking may appear surprising, because ascorbate is commonly regarded as an unstable compound (WHO Report, 1970). This is not true. If the pH is not alkaline, ascorbate is stable in solution, and no special precautions are needed to store vitamin C tablets. Certainly ascorbate is highly reactive, but only in the presence of oxidizing agents or specific enzymes. Ascorbate is reasonably stable in the cells of the fruits and vegetables that supply our dietary requirement (e.g. the unirradiated lemons of Fig. 14). A good deal of the loss during cooking is through leaching into the cooking water or canning liquid, rather than chemical breakdown.

Retention of thiamin, niacin and ascorbate in pineapple and tomato after canning is illustrated in Table 20. In some countries the option of eating fresh pineapples (with higher ascorbate content than canned pineapple) is not available. But pineapple preserved by canning is sterile and has a guaranteed shelf life of several years that cannot be matched by any irradiated fruit.

Table 20.

Retention of Vitamin Contents of Pineapple and Tomato After Canning; Amounts are mg per 100 g Edible Portion, and Expressed as % Compared to the Same Material Fresh.

Fruit	Thiamin mg	Thiamin %	Niacin mg	Niacin %	Ascorbate mg	Ascorbate %
Pineapple:						
+ sugar[a]	0.068	54	0.2	100	9	35
unsweetened[a]	0.060	48	0.2	100	7	27
+ sugar[b]	0.060	86	ND		7.7[d]	59
Tomato[a]	0.057	95	0.6	100	18	82
Tomato[b,c]	ND		0.6	86	9.6[d]	58

[a]Thomas and Corden (1970) [b]AGAL (1988)
[c]glass-house, not full sun [d]ascorbate + dehydroascorbate

* * * * * *

Table 21.

Nutrient Contribution Ranked by Consumption in the U.S.A.

Fruit/Vegetable	Rank	Fruit/Vegetable	Rank
Tomatoes	1	Sweet potatoes	10
Oranges	2	Peas	15
Potatoes	3	Spinach	18
Lettuce	4	Broccoli	21
Sweet corn	5	Lima beans	23
Bananas	6	Asparagus	25
Carrots	7	Cauliflower	30
Cabbage	8	Brussels sprouts	34
Onions	9	Artichokes	36

Because ripe tomatoes do not travel well, 'tougher' varieties are grown for the fresh fruit market and picked immature. It is universally appreciated that they lack flavour compared with home-garden varieties, which can be picked fully ripe. That is why canning is applied to select varieties of ripe tomatoes, which accordingly possess fully developed flavour, pigmentation, and maximum vitamin C content, ranging approximately from 16 to 21 mg per 100 g edible portion (Rick, 1978). Even if only 58% of the ascorbate survives canning (Table 20), this result is preferable to that for green tomatoes picked prematurely then irradiated. These remain green, and thereafter their ascorbate content can only decline from an initial value around 9 mg per 100 g (Maxie and Sommer, 1968).

The summer-grown tomatoes of California are more likely to resemble the earlier Australian sample of Table 20. The substantial consumption of canned and processed tomatoes in the USA indicates that a great many Americans obtain a high proportion of their vitamin C requirement from tomatoes (Table 21). This ranking was determined by M. A. Stevens more than 10 years ago (Rick, 1978), and is likely to still hold true for tomatoes.

Irradiated tomatoes could not fulfil the nutritional role presently met by canned tomatoes, even if they were irradiated ripe instead of green.