Attachment 42b

OCLC 1222940869 **No holdings in XXX - 171 other holdings**

Books

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Type	a	ELvl		Srce	d	Audn		Ctrl		Lang	eng		
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Desc	a	Ills		Fest	0	DtSt	s	Dates	2021,				

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037 9780128214640 $b Ingram Content Group
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082 04 664 $2 23
090 $b
245 00 Food structure and functionality / $c edited by Charis M. Galanakis.
260 London : $b Academic Press, $c 2021.
300 1 online resource
336 text $b txt $2 rdacontent
337 computer $b c $2 rdamedia
338 online resource $b cr $2 rdacarrier
500 Includes index.
650 0 Food $x Composition.
650 7 Food $x Composition. $2 fast $0 (OCoLC)fst00930485
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655 4 Electronic books.
700 1 Galanakis, Charis M.
776 08 $i Print version: $z 9780128214640
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Attachment 42c

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Public Catalog

Copyright Catalog (1978 to present)
Search Request: Left Anchored Title = food structure and functionality
Search Results: Displaying 1 of 1 entries

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Labeled View

Food Structure and Functionality.

Type of Work: Text
Registration Number / Date: TX0009034703 / 2021-02-26
Application Title: Food Structure and Functionality.
Title: Food Structure and Functionality.
Description: Book, 253 p.
Copyright Claimant: Elsevier Inc., Transfer: by written agreement with editors and all individual contributors. Address: 1600 John F Kennedy Blvd, Suite 1800, Philadelphia, PA, 19103, United States.
Date of Creation: 2020
Date of Publication: 2020-11-19
Nation of First Publication: United States
Authorship on Application: Charis Galanakis; Domicile: Greece; Citizenship: Greece. Authorship: text.

Pre-existing Material: permissioned materials.
Basis of Claim: text.
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Copyright Note: Basis for Registration: collective work.
ISBN: 9780128214534
Names: Galanakis, Charis
Elsevier Inc.

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Attachment 43a

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

MARC Bibliographic Record

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Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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072    _0    $aQ000

082    04    $a613.203$221

090          $aTX349$b.E47 2003

245    00    $aEncyclopedia of food sciences and nutrition /$ceditor-in-chief Benjamin Caballero,
             editors, Luiz C. Trugo and Paul M. Finglas.

250          $aSecond edition.

264    _1    $aAmsterdam ;$aBoston :$bAcademic,$c[2003]
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Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

264 _4 $c©2003

300 $a10 volumes :$billustrations, maps ;$c28 cm

336 $atext$btxt$2rdacontent

337 $aunmediated$bn$2rdamedia

338 $avolume$bnc$2rdacarrier

500 $aPrevious ed.: published as Encyclopedia of food science, food technology and nutrition. 1993.

504 $aIncludes bibliographical references and index.

505 0_ $av.1. A-Bro -- v. 2. Bro-Cla -- v. 3. Cle-End -- v. 4. Ene-Fru -- v. 5. Fru-I -- v. 6. J-M -- v. 7. N-Pre -- v. 8. Pre-Soy -- v. 9. Soy-V -- v. 10. W-Z, Index.

650 _0 $aNutrition$vEncyclopedias.

650 _0 $aFood$xComposition$vEncyclopedias.

700 1_ $aCaballero, Benjamin.

700 1_ $aTrugo, Luiz C.

700 1_ $aFinglas, Paul M.

994 $aX0$bGZM

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

997 $aMARCIVE

MMS IDs

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Attachment 43b

OCLC 52424395 **No holdings in XXX - 284 other holdings**

Books

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060 10 QU 13 $b E558 2003
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072 7 lw
082 04 613.203 $2 21
084 44.21 $2 bcl
084 VN 8000 $2 rvk
084 ZE 4000 $2 rvk
090 $b
245 00 Encyclopedia of food sciences and nutrition / $c editor-in-chief, Benjamin Caballero ; editors, Luiz C. Trugo, Paul M. Finglas.
250 2nd ed.
260 Amsterdam ; $a New York : $b Academic Press, $c ©2003.
300 10 volumes : $b illustrations
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
500 Revised edition of: Encyclopedia of food science, food technology and nutrition. 1993.
504 Includes bibliographical references and index.
505 0 v. 1. A-Bro -- v. 2. Bro-Cla -- v. 3. Cle-End -- v. 4. Ene-Fru -- v. 5. Fru-I -- v. 6. J-M -- v. 7. N-Pre -- v. 8. Pre-Soy -- v. 9. Soy-V -- v. 10. W-Z ; Index.
506 Access limited to subscribing institutions.
520 0 The success of the Encyclopedia of Food Science, Food Technology and Nutrition published by Academic Press in 1993 has led to this nine-volume set renamed the Encyclopedia of Food Sciences and Nutrition. This set is a substantial and up-to-date source of information in the areas of food science and nutrition, and is more global in coverage than its predecessor. An expansive indexing and cross-referencing system facilitates the location of information by keyword or text content. Every food scientist and nutritionist should have access to this stellar reference work.
650 0 Nutrition $v Encyclopedias.
650 0 Food $x Composition $v Encyclopedias.
650 0 Nutrition.
650 0 Food.
650 0 Food industry and trade $v Encyclopedias.
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650 12 Nutritional Physiological Phenomena $0 (DNLM)D009747
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700 1 Caballero, Benjamin.
700 1 Trugo, Luiz C.
700 1 Finglas, Paul M.
730 0 Encyclopedia of food science, food technology and nutrition.
856 41 $3 MacEwan University Access $u http://www.sciencedirect.com/science/referenceworks/9780122270550 $z (Unlimited Concurrent Users)
856 41 $3 ScienceDirect $u http://www.sciencedirect.com/science/referenceworks/012227055X
856 41 $3 Table of contents $u http://catdir.loc.gov/catdir/toc/els031/2002114339.html

Attachment 43c

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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LC control no.: sh2010104090
LCCN Permalink: https://lccn.loc.gov/sh2010104090
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Attachment 43d

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

The Library's Main Reading Room will close to researchers earlier than usual, at 5:00pm, on Thurs., Nov. 3. All other reading rooms will be open according to their daily schedules.

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LCCN Permalink: https://lccn.loc.gov/sh85042997
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360 __ |**i** subdivisions |**a** Dictionaries |**i** or |**a** Encyclopedias |**i** under subjects
450 __ |**a** Books of knowledge
450 __ |**a** Cyclopedias
450 __ |**a** Dictionaries
450 __ |**a** Encyclopedias and dictionaries, English
450 __ |**a** Knowledge, Books of
450 __ |**a** Subject dictionaries
550 __ |**w** g |**a** Reference books
680 __ |**i** Here are entered comprehensive reference works in the English language consisting of explanatory articles arranged alphabetically or topically and not limited to a specific topic. Works of this type in other languages are entered under headings of the type Encyclopedias and dictionaries, [language], e.g. |**a** Encyclopedias and dictionaries, French; Encyclopedias and dictionaries, Spanish. |**i** Works about encyclopedias and dictionaries in general, and works about English-language encyclopedias and dictionaries, are entered under |**a** Encyclopedias and dictionaries |**i** with further subdivision, e.g. |**a** Encyclopedias and dictionaries--History and criticism. |**i** Works

consisting of comprehensive alphabetical lists of the words of a specific language, usually with definitions, are entered under the heading for the language with subdivision Dictionaries, e.g. |**a** English language--Dictionaries. |**i** Works about dictionaries of a specific language are entered under the heading for the language with subdivision Lexicography, e.g. |**a** English language--Lexicography.

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953 __ |**a** xx00 |**b** td14

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Attachment 43e

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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906 __ |**t** 8888 |**u** te00 |**v** 0
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URL: http://authorities.loc.gov/
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Attachment 44a

MARC Bibliographic Record

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Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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049 $aGZMA

050 00 $aQD71.5$b.E53 2005

082 00 $a543.03$222

245 00 $aEncyclopedia of analytical science /$ceditors, Paul Worsfold, Alan Townshend, Colin Poole.

250 $aSecond edition.

264 _1 $aAmsterdam ;$aBoston :$bElsevier Academic Press,$c[2005]

264 _4 $c©2005

300 $a10 volumes :$billustrations (some color) ;$c28 cm

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Case No.: IPR2023-00209
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505 0_ $av. 1. A-Chem -- v. 2. Chem-E -- v. 3. F -- v. 4. G-I -- v. 5. K-Mer -- v. 6. Mic-O -- v. 7. P-Q -- v. 8. R-S -- v. 9. T-Z -- v. 10. Index.

504 $aIncludes bibliographical references and index.

650 _0 $aAnalytical chemistry$vEncyclopedias.

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650 _6 $aChimie analytique$vDictionnaires anglais.

700 1_ $aWorsfold, Paul.

700 1_ $aTownshend, A.$q(Alan),$d1939-

700 1_ $aPoole, C. F.

856 42 $3Book review (E-STREAMS)$uhttp://www.e-streams.com/es0808/es0808%5F4221.html

856 42 $3Publisher description$uhttp://www.loc.gov/catdir/enhancements/fy0619/2004104444-d.html

938 $aBaker & Taylor$bBKTY$c5015.00$d5015.00$i0127641009$n0004410286$sactive

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Attachment 44b

OCLC 61287955 **No holdings in XXX - 70 other holdings**

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670 __ |**a** McGraw-Hill dictionary of chemistry, 2003 |**b** (analytical chemistry: The branch of chemistry dealing with techniques which yield any type of information about chemical systems)

670 __ |**a** Analytical chemistry, via American Chemical Society website, Aug. 10, 2020 |**b** (Analytical chemistry is the science of obtaining, processing, and communicating information about the composition and structure of matter. In other words, it is the art and science of determining what matter is and how much of it exists. In 2012 (salary survey data), analytical chemistry was the most popular field of work for ACS chemists. Analytical chemists use their knowledge of chemistry, instrumentation, computers, and statistics to solve problems in almost all areas of chemistry and for all kinds of industries. For example, their measurements are used to assure the safety and quality of

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670 __ |**a** Webster's new world college dictionary, ©2010, via Collins dictionary website, Aug. 10, 2020 |**b** (analytical chemistry: a branch of chemistry that deals with the identification of compounds and mixtures (qualitative analysis) or the determination of the proportions of the constituents (quantitative analysis): techniques commonly used are titration, precipitation, spectroscopy, chromatography, etc.)

670 __ |**a** Dictionary.com, Aug. 10, 2020 |**b** (analytical chemistry: the subdivision of chemistry dealing with the qualitative and quantitative determination of chemical components of substances)

675 __ |**a** A dictionary of chemistry, 2020

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JOURNAL ARTICLE

Evidence that the Plant Host Synthesizes the Heme Moiety of Leghemoglobin in Root Nodules[1] FREE

Maria A. Santana, Kaarina Pihakaski-Maunsbach, Niels Sandal, Kjeld A. Marcker, Alison G. Smith
Author Notes

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Abstract

Although it is well established that the plant host encodes and synthesizes the apoprotein for leghemoglobin in root nodules, the source of the heme moiety has been uncertain. We recently found that the transcript for coproporphyrinogen III oxidase, one of the later enzymes of heme synthesis, is highly elevated in soybean (*Glycine max*L.) nodules compared with roots. In this study we measured enzyme activity and carried out western-blot analysis and in situ hybridization of mRNA to investigate the levels during nodulation of the plant-specific coproporphyrinogen oxidase and four other enzymes of the pathway in both soybean and pea (*Pisum sativum* L.). We compared them with the activity found in leaves and uninfected roots. Our results demonstrate that all of these enzymes are elevated in the infected cells of nodules. Because these are the same cells

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that express apoleghemoglobin, the data strongly support a role for the plant in the synthesis of the heme moiety of leghemoglobin.

Issue Section: PLANT-MICROBE AND PLANT-INSECT INTERACTIONS

The major tetrapyrrole synthesized in plants is chlorophyll, which in leaf tissue may be present at 2.5 μmol/g fresh weight. In contrast, the levels of other tetrapyrroles such as siroheme, phytochromobilin, and heme are much lower, with an estimated 2 nmol/g fresh weight for mitochondrial heme. However, in leguminous root nodules, levels of heme may be elevated a few hundred-fold. Nodules are unique, specialized organs that are the result of a symbiotic association between plants of the family *Leguminosae* and soil bacteria of the genera *Sinorhizobium*,*Rhizobium*, *Bradyrhizobium,* and*Azorhizobium*. The bacteria are present in the infected plant cell surrounded by the peribacteroid membrane, which is derived from the plant cell membrane. There they differentiate into bacteroids and express the enzyme nitrogenase, which enables them to fix atmospheric dinitrogen, thus allowing the plant host to grow without external reduced nitrogen. Nitrogenase is oxygen-sensitive, but the vigorously respiring bacteroids require an adequate supply of oxygen. This is achieved by the presence of leghemoglobin, which facilitates oxygen diffusion to the endosymbiont. Leghemoglobin has been immunolocalized to the cytosol of the infected plant cell, and is absent from the bacteroid and peribacteroid space (for review, seeAppleby, 1984).

It is well established that the plant host encodes the gene for the leghemoglobin apoprotein (Jensen et al., 1981), but the source of the heme moiety has been the subject of much debate. Early biochemical work found that plant nodule tissue could not synthesize heme, whereas the rhizobia both made and exported heme (Cutting and Schulman, 1969). Similarly, the bacteroid fraction of soybean (*Glycine max*L.) nodules contained detectable levels of the enzyme ALA synthase, but the plant cytosol had none (Nadler and Avissar, 1977). ALA is the first committed precursor of all cellular tetrapyrroles (Fig.1). The conclusion drawn from these studies was that the bacterial endosymbiont was responsible for the synthesis of the heme group of leghemoglobin, leading to proposals that leghemoglobin synthesis is a means of intimate contact between the plant and bacterial host (Appleby, 1984; Haaker, 1988).

Fig. 1.

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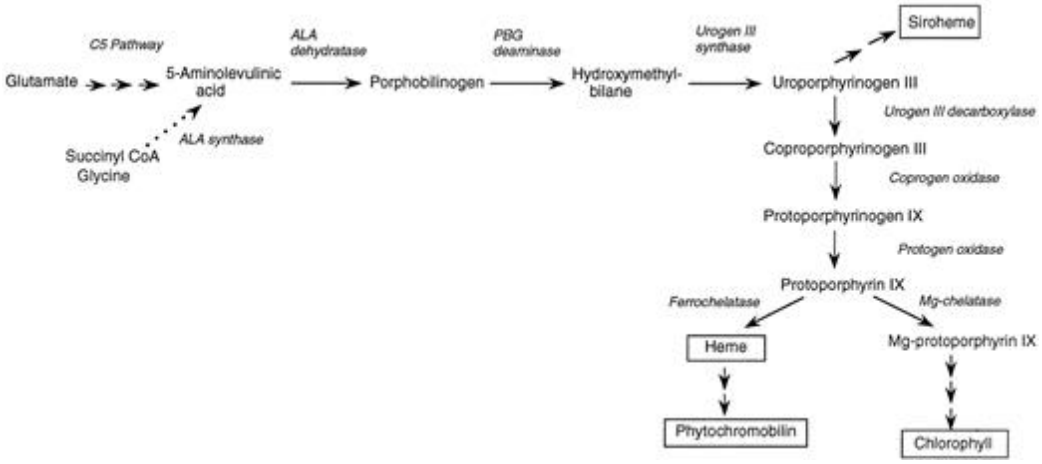

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Pathway of tetrapyrrole synthesis in higher plants, showing the major end products (boxed) and relevant intermediates and enzymes. In plants, algae, and most bacteria, the first committed precursor ALA is synthesized from glutamate in the C5 pathway in three steps. However, in rhizobial species (as well as in certain other bacteria, animals, and fungi) ALA is synthesized in a single step by ALA synthase (dotted line). Urogen, Uroporphyrinogen.

However, the conclusions were based on erroneous assumptions about plant heme biosynthesis (for review, see O'Brian, 1996). In particular, it is now known that plant cells synthesize ALA by a completely different route, namely from glutamate via the so-called C5 pathway involving three enzymes (for review, see Kannangara et al., 1988). Glutamate-dependent ALA synthesis has been shown to be present at much higher levels in soybean nodules than in uninfected roots (Sangwan and O'Brian, 1992), whereas bacterial ALA synthase activity was essentially the same in nodules and in free-living bacteria. Furthermore, the plant gene for one of the C5 pathway enzymes, GSA aminotransferase, is induced during nodulation, concomitantly with the increase in enzyme activity (Sangwan and O'Brian, 1993; Frustaci et al., 1995). The same group also investigated the next enzyme in the pathway, ALA dehydratase. They found that, although message levels were relatively high in roots, there was no detectable protein. However, in nodules both protein level and enzyme activity are markedly increased (Kaczor et al., 1994). The results of both these studies suggest that the plant host responds during nodulation to the need for increased heme biosynthesis.

We have reported the isolation and characterization of a soybean cDNA that is strongly induced during nodulation, with a time course comparable to the increase in leghemoglobin (Madsen et al., 1993). This cDNA was identified as encoding coprogen oxidase, a later enzyme of tetrapyrrole synthesis (Fig. 1), because it showed considerable sequence similarity to the same enzyme isolated from yeast (Zagorec et al., 1988) and was able to complement a yeast mutant with a deletion in the coprogen oxidase gene (Madsen et al., 1993). It has since been

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shown to encode a protein with coprogen oxidase activity (M.A. Santana and A.G. Smith, unpublished data). The fact that it is induced in root nodules suggests, as for GSA aminotransferase and ALA dehydratase, that it plays a role in the requirement for increased heme synthesis in these organs. To investigate this further, we carried out a careful biochemical and molecular analysis of the levels of coprogen oxidase enzyme during nodulation and also examined four other enzymes of the pathway, namely ALA dehydratase, PBG deaminase, protogen oxidase, and ferrochelatase (Fig. 1). This paper presents our findings.

MATERIALS AND METHODS

Growth of Plant Material and Nodulation

Soybean (*Glycine max* L., cv Merrill) seeds were surface sterilized in hypochlorite and then soaked in water overnight. They were planted in trays containing sterile, lightweight, expanded clay aggregate. Pea (*Pisum sativum* L., cv Feltham First) seeds were surface sterilized and soaked in water for 1 to 2 h. They were then sown in Levington compost (Fisons, Beverly, MA). Plants were maintained in a greenhouse at approximately 25°C under a 16-h light, 8-h dark cycle or, alternatively, in complete darkness for the production of etiolated plants. After 2 weeks of growth, soybean plants were inoculated with *Bradyrhizobium japonicum* USDA110 and pea plants were inoculated with *Rhizobium leguminosum* bv*viciae* strain 3841. For growth under anaerobic conditions, 2-week-old soybean plants were placed in pots in which the roots were submerged in water continuously bubbled with nitrogen to saturate it. A set of control plants was maintained under the same conditions but bubbled with air.

Tissue extracts for enzyme assays and western analysis were prepared by grinding fresh material in 50 mm Hepes-NaOH, pH 8.2, 6 mm $MgCl_2$, and 5 mm 2-mercaptoethanol in a mortar with a pestle and a small amount of acid-washed sand and centrifuging at 13,000*g* for 10 min. They were stored at -70°C until needed.

Enzyme Assays and Analytical Methods

ALA dehydratase and PBG deaminase assays were carried out as described by Smith (1988), and coprogen oxidase and protogen oxidase were measured fluorimetrically as described by Smith et al. (1993). Ferrochelatase was assayed spectrofluorimetrically using deuteroporphyrin IX as the substrate by the method of Porra and Lascelles (1968). Alcohol

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dehydrogenase was assayed as described by Mohanty et al. (1993). All assays were carried out two to four times on at least two independent extractions. Chlorophyll was measured by the method of Arnon (1949). Protein was determined with a protein estimation kit (Bio-Rad) according to the manufacturer's instructions, using BSA as the standard. Heme was measured with a hemoglobin kit (Sigma), following the manufacturer's instructions.

Western Analysis

For western analysis, protein extracts (10–50 μg protein) were subjected to electrophoresis, as described by Laemmli (1970), on 12.5% polyacrylamide gels in the presence of SDS (1% [w/v] in the gel, 0.1% [w/v] in the electrode buffer), and then transferred to nitrocellulose membranes (Schleicher & Schuell) using a semidry blotting apparatus (Atto Corp., Tokyo). Proteins were visualized with Ponceau S and washed in TBS (20 mm Tris-HCl, pH 7.5, and 500 mm NaCl), and the nonspecific protein sites were blocked overnight with 3% nonfat, powdered milk in TBS. The blots were then challenged with antiserum raised against soybean coprogen oxidase (M.A. Santana and A.G. Smith, unpublished data) at a 1:2000 dilution in TBS containing 0.05% Tween 20 and 1% nonfat milk. Bound antibodies were visualized with goat anti-rabbit antibodies conjugated to alkaline phosphatase (Bio-Rad) according to the manufacturer's instructions.

In Situ Hybridization

Pea and soybean root nodules of various sizes were harvested 26 d after inoculation and fixed with FAA (3.7% formaldehyde, 5% acetic acid, and 50% ethanol) or with 4% para-formaldehyde and 0.25% glutaraldehyde in 50 mm sodium phosphate buffer (pH 7.2) for 4 h, dehydrated in graded ethanol and xylene series, and embedded in Paraplast. Sections (7 μm) were attached to poly-l-Lys-coated slides, deparaffinized with xylene, and rehydrated through a graded ethanol series. Cross-sections of nodules were hybridized with ^{35}S-labeled antisense or sense RNAs (see below), as described by van de Wiel et al. (1990) from a modification of the method of Cox and Goldberg (1988). Sections were pretreated with proteinase K, dehydrated, dried under a vacuum until they were coated with NTB2 nuclear emulsion (Kodak), and exposed for 7 to 40 d at 4°C. Slides were developed in D19 developer (Kodak) and fixed in Unifix (Kodak). Sections were stained with 0.25% toluidine blue, dehydrated, and mounted with DPX (BDH, Toronto, Ontario, Canada). They were viewed and photographed with an axioscope (Zeiss) equipped with dark-field and epipolarization optics on Fujicolor HG Super 100 film.

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Sense and antisense RNA probes were prepared from the pea ALA dehydratase cDNA clone pALAD209 (Boese et al., 1991; a gift from Dr. M. Timko, University of Virginia, Charlottesville), the pea PBG deaminase cDNA clone pPD1 (Witty et al., 1993), and the soybean coprogen oxidase cDNA clone pCOF (Madsen et al., 1993). RNA was transcribed in vitro from each of these clones using SP6 or T7 polymerase, as appropriate, in the presence of ^{35}S-UTP (1250 Ci/mmol, Amersham) and was degraded to about 150-nucleotide-long fragments before hybridization.

RESULTS

Induction of Coprogen Oxidase during Nodulation

The maximum level of coprogen oxidase mRNA was seen at about 3 weeks after infection (Madsen et al., 1993), just as the number of observable nodules starts to increase. This is also the time at which the rate of heme synthesis is maximal (Nadler and Avissar, 1977;Sangwan and O'Brian, 1992). However, the amount of transcript is not necessarily a direct indication of the amount of enzyme; therefore, we investigated this with western-blot analysis and a determination of enzyme activity over the same time course of nodulation. Figure2 shows the results of a representative experiment. The levels of activity were virtually undetectable in uninfected roots when the fluorimetric assay for coprogen oxidase was used (Labbe et al., 1985), although with the more sensitive radioactive assay (Elder and Evans, 1978), it was possible to measure activity reproducibly (data not shown). However, within 1 week of inoculation with *B. japonicum,* coprogen oxidase activity started to increase markedly, reaching a maximum at 3 weeks (Fig. 2A). The increase in specific activity was 27-fold compared with uninfected roots. Because there was also an increase in protein during the development of nodules, if the activity is expressed per gram fresh weight, then the increase is even more dramatic at 250-fold, although the shape of the curve over the whole time course is essentially the same (data not shown). Thereafter, coprogen oxidase activity declined, but there was considerable activity even at 7 weeks (when the nodules are effectively senescent), more in fact than was found in green leaves (see below).

Fig. 2.

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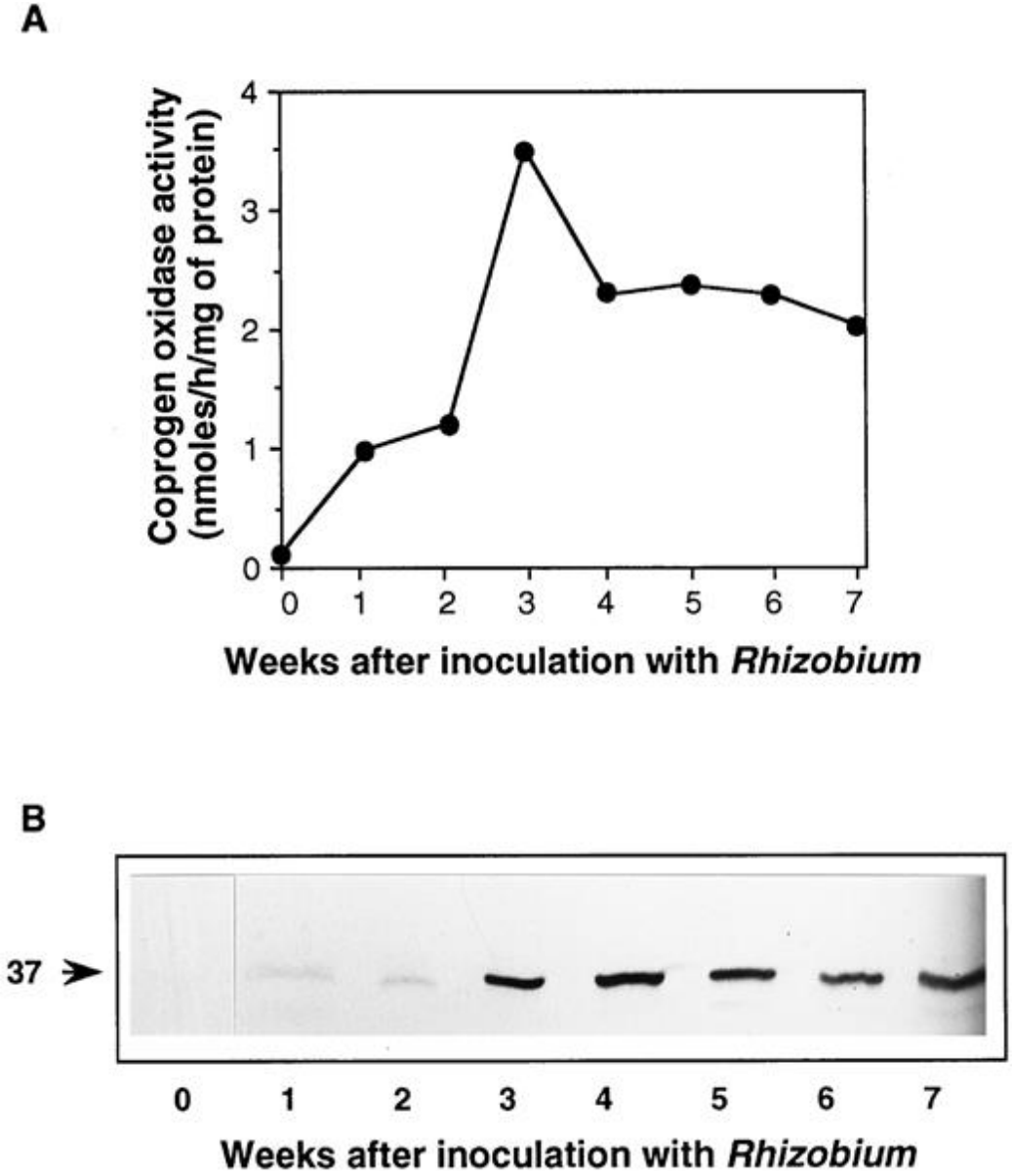

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Changes in coprogen oxidase during the development of soybean root nodules. A, Coprogen oxidase activity measured with the fluorimetric assay. Once determinate nodules were visible (after 2–3 weeks), these were removed from the roots, but the samples before this included some root material. The sample before inoculation was root alone. Each point is the mean of three assays of the same sample extract; the variation between assays was less than 10%. B, Western-blot analysis of coprogen oxidase on the same samples assayed in A. Soluble proteins (10 µg) were separated by SDS-PAGE, blotted onto nitrocellulose, and then challenged with polyclonal antibodies raised against recombinant soybean coprogen oxidase. Bound antibodies were visualized with alkaline phosphatase-linked second antibody followed by colorimetric detection. The arrow indicates the 37-kD coprogen oxidase protein.

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Because the assays were carried out on extracts from whole, unfractionated nodules, it was not possible to determine whether the enzyme activity was associated with the plant host or the bacterial symbiont. We therefore carried out western-blot analysis. Samples of the

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soluble protein extracts were subjected to electrophoresis and transferred to nitrocellulose. The blot was then challenged with antibodies raised against the 37-kD soybean coprogen oxidase (M.A. Santana and A.G. Smith, unpublished data). These antibodies do not cross-react with a protein of similar size in extracts of free-living*B. japonicum* (data not shown). It can be seen (Fig. 2B) that during the first 4 weeks there was a parallel increase in the levels of enzyme protein, although there was not such an obvious decline in the older nodules. The amount of coprogen oxidase protein was estimated by densitometry to be 14 times greater in 3-week-old nodules than in uninfected roots. Although this figure shows that no immunoreactive protein was visible in the uninfected roots, when 3 times more protein was used (30 μg), a band was detected (Fig.3, lane 0). From these experiments, it would appear that the nodule-induced increase in expression of the soybean coprogen oxidase gene results directly in an increase in plant-specific coprogen oxidase protein, with a concomitant increase in enzyme activity within the nodule. Similar time courses of induction were observed after western-blot analysis of two of the earlier enzymes of the pathway, GSA aminotransferase (Sangwan and O'Brian, 1993;Frustaci et al., 1995) and ALA dehydratase (Kaczor et al., 1994).

Fig. 3.

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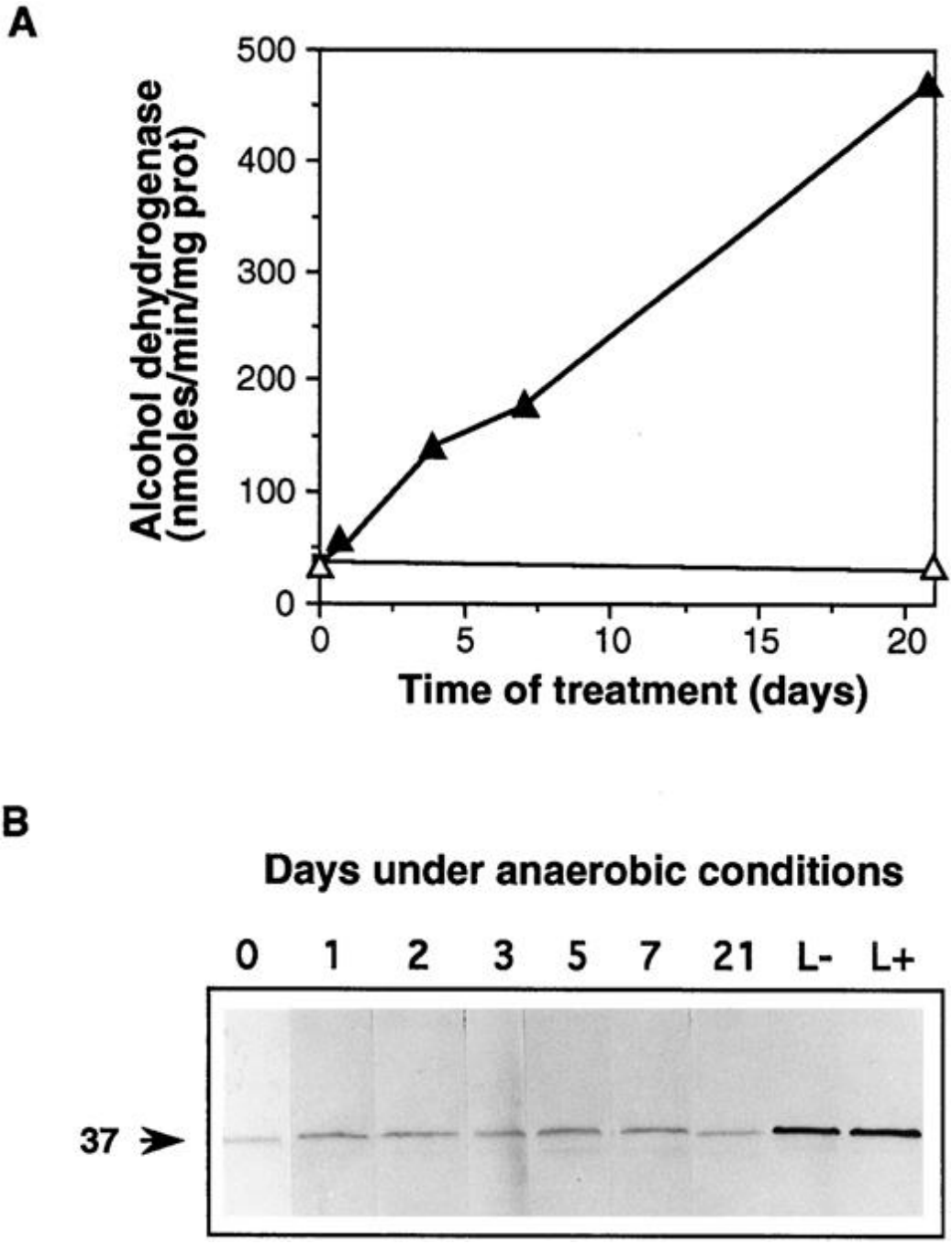

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Effect of anaerobiosis on soybean roots. Two-week-old soybean plants were placed in pots so that the roots were submerged in water through which either air or nitrogen was bubbled. A, Activity of alcohol dehydrogenase in roots under aerobic (▵) or anaerobic (▲) conditions. The increase in alcohol dehydrogenase in the latter samples confirmed that the roots were anaerobic (Russell et al., 1990). Each point is the mean of two assays carried out on the same extract. Levels of coprogen oxidase activity remained essentially undetectable in these roots throughout the treatment. B, Western analysis of coprogen oxidase. Soluble protein (30 µg) from roots grown under anaerobic conditions (0–21 d) or leaves from plants grown aerobically (L−) or anaerobically (L+) for 21 d were challenged with coprogen oxidase antibodies as described in the legend of Figure2. prot, Protein.

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Correlation between Coprogen Oxidase Activity and Leghemoglobin Synthesis

The observation that plant coprogen oxidase increases during nodulation suggests that it has a role in enhanced heme biosynthesis, presumably for leghemoglobin. To investigate this further, we determined the levels of heme in different-aged nodules and calculated the rate of heme synthesis over the period 3 to 4 weeks postinoculation to be 0.73 nmol h^{-1} g^{-1}fresh weight (Table I). For comparison, the rate of chlorophyll synthesis in soybean leaves was determined, both during greening of dark-grown etiolated plants and during leaf expansion of light-grown plants, and both were found to be much higher than that of heme synthesis in nodules. In contrast, coprogen oxidase activity was much greater in the nodules than in the leaves. This was also seen in the western analysis, in which the amount of coprogen oxidase per unit protein was estimated by densitometry to be 5 times greater in 3-week-old nodules (Fig. 2B, lane 3) compared with light-grown leaves (Fig. 3B, lane L).

Table I. Rates of tetrapyrrole synthesis and levels of tetrapyrroles in soybean tissues

Tissue	Coprogen Oxidase	Rate of Tetrapyrrole Synthesis	Heme	Chlorophyll
	nmol h^{-1} g^{-1} fresh wt		nmol g^{-1} fresh wt	
Nodules 21 d after infection	108.2 ±9.2	—	58.6 ±8.9	
Nodules 28 d after infection	81.4 ±6.2	0.73	181.5 ± 10.3	
24-h Greened leaves	35.0 ±4.8	—		388.9 ±23.0
48-h Greened leaves	15.9 ±1.6	26.9		955.6 ±16.5
Newly emerged leaves	38.9 ±1.8	—		2330.0 ± 58.9
Fully expanded leaves	16.6 ±0.2	3.3		2890.0 ± 89.5

Soybean plants were grown as described in Methods. For the measurement of heme synthesis during nodulation, they were grown in a greenhouse for 14 d before infection with *B. japonicum.* For chlorophyll synthesis during greening, plants were grown in complete darkness for 7 d and then exposed to continuous illumination. The rate was determined between 24 and 48 h, in the exponential phase. For chlorophyll synthesis during leaf expansion of light-grown plants, samples of leaves were taken at the newly emerged stage and then

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again when they were fully expanded (7 d later). The enzyme activity values are the means ± se of four samples, and those for heme and chlorophyll are the means of three samples.

The fact that the activity of coprogen oxidase in nodules was much greater than that required to account for the rate of heme synthesis might mean that the induction of coprogen oxidase activity is unrelated to the need to synthesize more heme and is due to some other factor. One possibility is that the low free-oxygen content within the nodule is responsible for the induction. Coprogen oxidase uses molecular oxygen as one of its substrates, and in yeast the enzyme is induced by anaerobiosis (Zagorec and Labbe-Bois, 1986). It has been proposed that this induction in yeast is to ensure a large excess of the enzyme to scavenge any available oxygen so that heme synthesis may continue. We therefore investigated the effect of anaerobiosis on levels of coprogen oxidase by growing soybean roots in water through which either air or nitrogen was continuously bubbled. Root samples were taken after 1, 3, 5, 7, and 21 d of treatment, when plants under anaerobic conditions showed severe chlorosis of young leaves, growth retardation, and early senescence of mature leaves. There was considerable induction of alcohol dehydrogenase activity in the roots compared with those grown in air-saturated water (Fig. 3A), demonstrating that they were indeed under anaerobic stress (Russell et al., 1990). In contrast, coprogen oxidase activity remained essentially undetectable with the fluorimetric assay. This is also reflected in the western blot (Fig.3B), in which the levels of enzyme protein remained unchanged, considerably less than that present in leaves, either those from anaerobically treated plants (L+) or from controls (L-). In nodules there was 3 to 10 times more coprogen oxidase activity and protein than in green leaves (Table I). Similar results were obtained when a suspension culture of soybean cells was grown under anaerobic conditions. Although alcohol dehydrogenase activity was induced from 63 to 1385 nmol min^{-1} mg^{-1}protein after 8 d, as observed previously by Mohanty et al. (1993), coprogen oxidase activity remained essentially undetectable throughout the anaerobic treatment. Therefore, it is unlikely that the induction of coprogen oxidase in nodules is due to the anaerobic conditions but rather is due to a response to the need for increased heme synthesis.

Levels of Other Tetrapyrrole Synthesis Enzymes in Root Nodules

To investigate this further, the activities of other heme synthesis enzymes during the nodulation process were determined. Figure4 shows the results of a representative experiment expressed per gram fresh weight; essentially similar shaped curves were seen for enzyme-specific activity (data not shown). The activities of all of the enzymes followed very similar time courses over the 5-week period: in each case there was an increase in activity starting at about 14 d, which was when the first nodules were visible on the roots. The levels peaked at 21 d, concomitantly with the initial appearance of leghemoglobin, and were more

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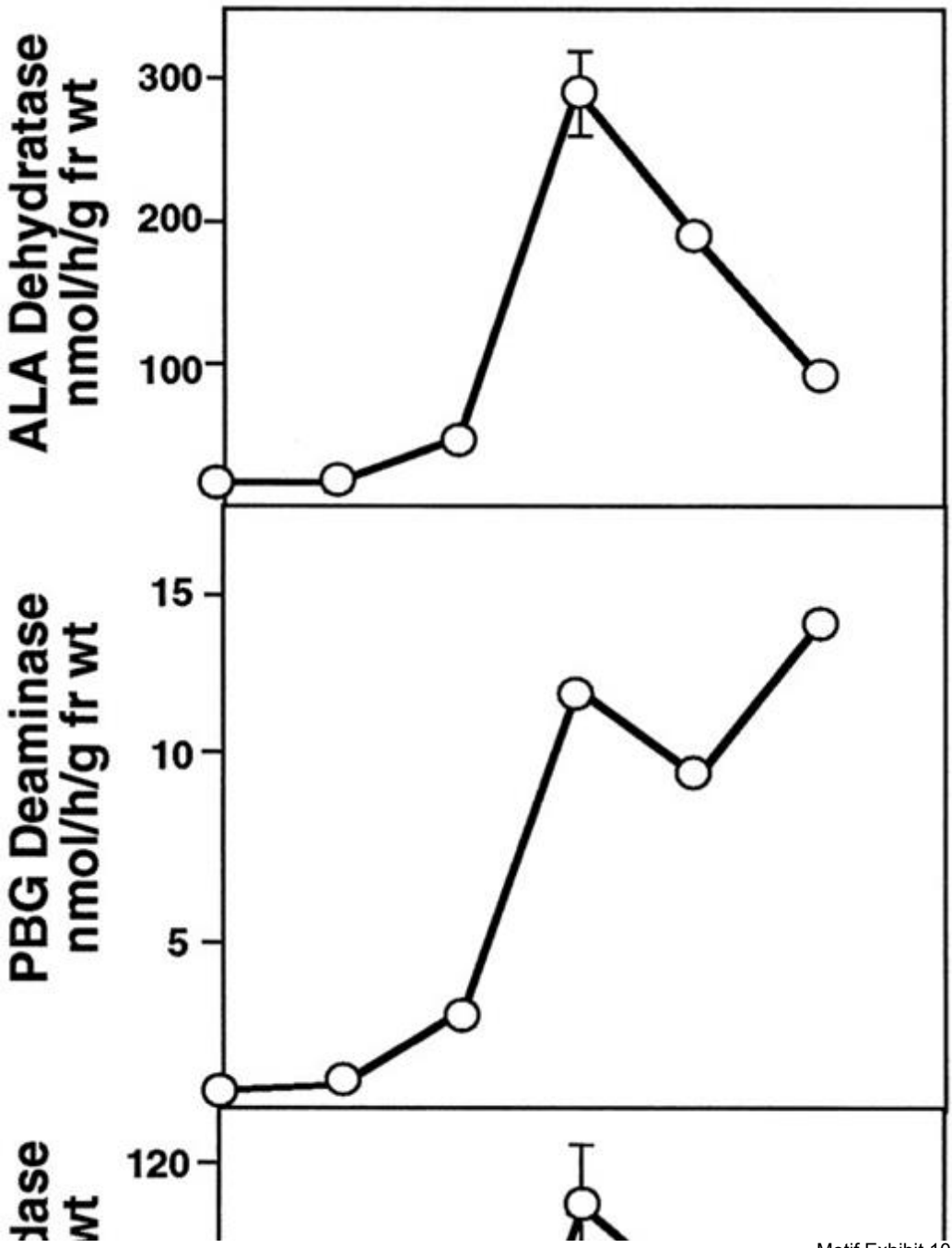
than sufficient to account for the rate of heme synthesis in nodules during this period. The stimulation of the first three enzymes, ALA dehydratase, PBG deaminase, and coprogen oxidase, was about 17-fold, whereas it was only 3- to 4-fold for protogen oxidase and ferrochelatase, respectively, probably because the activities of the latter two enzymes were relatively high, even in uninfected roots. This increase in enzyme activity was specific to the nodule tissue, because the activities in the roots of the nodulated plants were essentially unaltered compared with uninfected plants (TableII). When the levels in nodules were compared with those in expanded green leaves, differences between the enzymes were apparent. Both ALA dehydratase and PBG deaminase activities were higher in leaves than in nodules, whereas for the two oxidases and ferrochelatase the reverse was true.

Fig. 4.



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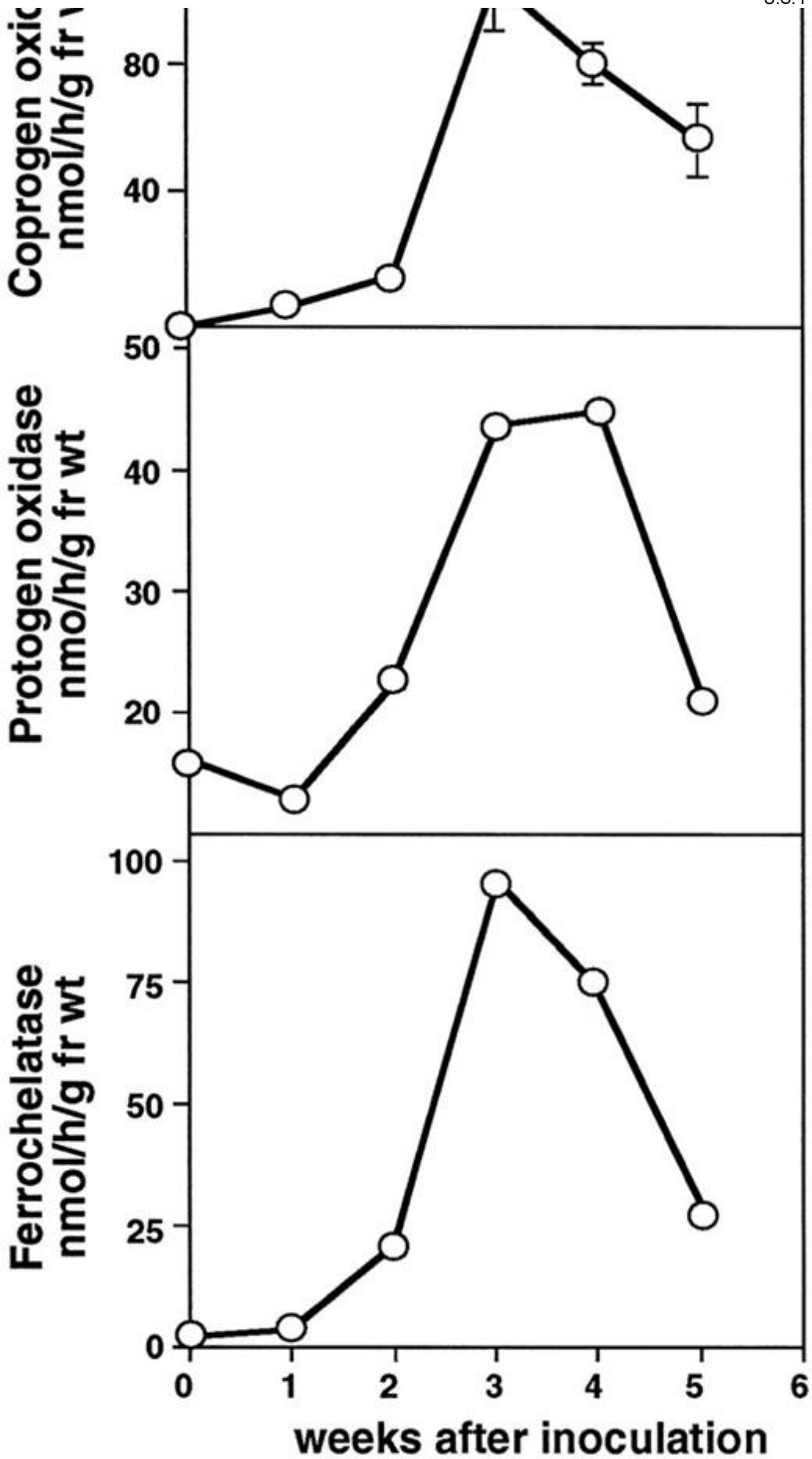

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Activity of heme biosynthesis enzymes during the development of soybean root nodules. Samples of root nodule were taken as described in the legend of Figure 2 and assayed for the different enzyme activities. Each value is the mean of four replicate assays, except ferrochelatase, which is the mean of two replicates. The variation in assay values were in general less than 10%. This is a representative experiment; essentially similar time courses were observed on several occasions. fr wt, Fresh weight.

Table II. Activities of heme synthesis enzymes in soybean tissues

Heme Synthesis Enzyme	Before Inoculation		Three Weeks after Inoculation	
	Expanded leaves	Roots (uninfected)	Roots (infected)	Nodules
	nmol h^{-1} g^{-1} fresh wt			
ALA dehydratase (3)	317.0 ±19.0	16.7 ±2.8	18.0 ±2.0	291.0 ±3.3
PBG deaminase (3)	34.0 ±2.71	0.594 ±0.04	0.624 ±0.04	10.9 ±0.38
Coprogen oxidase (4)	16.6 ±0.21	n.d.[1-a]	n.d.	108.2 ±9.2
Protogen oxidase (4)	15.9 ±0.11	15.9 ±2.2	18.4 ±0.25	43.5 ±6.0
Ferrochelatase (2)	55.7	22.8	39.2	95.8

The levels of heme synthesis enzymes were determined in roots and expanded leaves of 2-week-old soybean plants and then again in nodules and roots 3 weeks after inoculation with *B. japonicum.* The values are the means ± se of the number of samples indicated in parentheses. Because four molecules of PBG are required for each tetrapyrrole molecule, in general, in most plant tissues examined the ALA dehydratase rate is much higher than that of the other enzymes of the pathway (Smith, 1986, 1988).

F1-a n.d., Not detectable.

To determine whether this increase in heme synthesis enzymes was a universal feature of nodule physiology or unique to soybean, enzyme activities were determined during nodulation of pea roots with *R. leguminosarum* bv *viciae*. TableIII presents the activity of ALA dehydratase, PBG deaminase, coprogen oxidase, and protogen oxidase in uninfected pea roots and in nodules 21 d after infection. For each enzyme, the activity was enhanced in the nodules, and as in soybean, both coprogen and protogen oxidase activities were greater in nodules than in green leaves.

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Table III. Activity of heme synthesis enzymes in pea tissues

Heme Synthesis Enzyme	Expanded Leaves	Uninfected Roots	Three-Week-Old Nodules

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		nmol h^{-1} g^{-1} fresh wt	
ALA dehydratase	2400.0	30.1	225.0
PBG deaminase	191.8	2.99	23.5
Coprogen oxidase	11.2	n.d.	55.5
Protogen oxidase	17.1	n.d.	42.5

Pea plants were grown under a 16-h light, 8-h dark cycle as described in Methods. After 14 d, root and leaf samples were taken for assay, and the plants were inoculated with *R. leguminosarum* bv. *viciae.* Nodules (approximately 2 mm in diameter) were harvested after 3 weeks. The activities are the means of two determinations. The variation between samples was less than 10%. n.d., Not detectable.

In Situ Localization of Transcripts for Heme Synthesis Enzymes

Although the measurement of enzyme activities in nodules showed that the heme synthesis enzymes were elevated, as for coprogen oxidase, it is not possible to distinguish between the bacterial and plant enzymes when measuring enzyme activity alone. However, plant coprogen oxidase transcripts are increased in nodules (Madsen et al., 1993), as are transcripts of plant ALA dehydratase (Kaczor et al., 1994) and plant GSA aminotransferase (Sangwan and O'Brian, 1993; Frustaci et al., 1995). We have found that transcripts of PBG deaminase also increase during nodule development (data not shown). We have now studied the distribution of transcripts by in situ hybridization of three enzymes for which we had suitable cDNA clones. As well as demonstrating the increase in plant-specific message levels, it also provided information about the spatial location of the transcripts within the nodule.

The probes used were the cDNAs for soybean coprogen oxidase (Madsen et al., 1993), pea PBG deaminase (Witty et al., 1993), and pea ALA dehydratase (Boese et al., 1991). Antisense RNAs for each of these clones were prepared and hybridized to sections of soybean root nodules of 1 to 2 mm (3–4 weeks after infection), where the maximum induction of enzyme activity had been observed. In nodules of this size, the meristematic region has already differentiated into nodular tissue because soybean nodules are of the determinate type. The largest part of such a nodule is composed of central tissue containing mainly infected cells, which are surrounded by the nodule inner cortex and the outermost cortex (outer cortex). Sense RNAs were used as controls.

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With a ^{35}S-labeled antisense probe for ALA dehydratase, the transcript was localized in the central tissue of the nodule (Fig. 5, A and B), mainly in infected cells, with a gradient of expression in the infected zone of the central tissue. An increased signal was also present in

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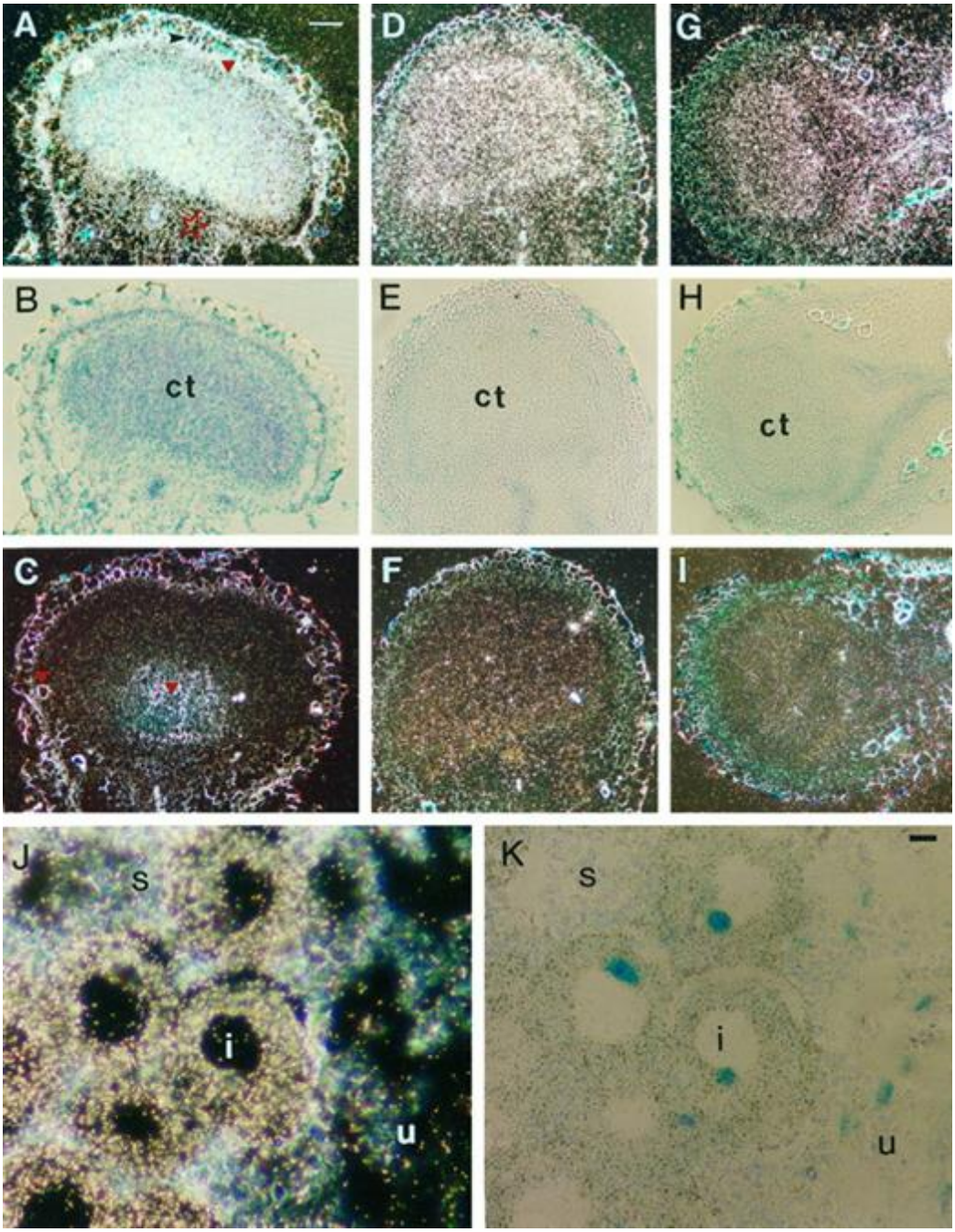
the nodule inner cortex (Fig. 5, A and B, red triangle), but a weak signal was visible in the uninfected cells of the outer cortex (Fig. 5A, red star). This was concluded from the light-microscope observations made at a higher magnification. No hybridization was obtained when the ^{35}S-labeled sense probe was used (Fig. 5C). Some thick-walled schlerenchyma cells, lignified xylem cells, and starch grains were refractive in dark-field microscopy (Fig. 5C, red triangles).

Fig. 5.



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Localization of ALA dehydratase (A–C), PBG deaminase (D–F), and coprogen oxidase (G–I) transcripts in longitudinal sections of soybean nodules using ^{35}S-labeled probes. A, C, D, F, G, and I, Dark-field micrographs in which silver grains are visible as white dots. B, E, and H, Bright-field micrographs of the same sections that show the antisense RNAs pictured in A, D, and G. An intense signal in the infected cells of the central tissue (ct) is observed in A, D, and G. Increased expression in the nodule inner cortex (red triangle in A) and weak expression in the outer cortex (red star in A) are apparent. A black arrowhead points to the endodermis in A. No specific signal was detectable in the micrographs probed with the sense transcripts (C, F, and I). Thick-walled schlerenchyma cells, lignified xylem cells, and starch grains appear yellowish-white (red triangles in C). Bar = 100 µm. J and K, Localization of PBG deaminase transcripts in longitudinal section of the root nodule of pea using the ^{35}S-labeled probe. Dark-field micrograph (J) shows that transcripts are localized in the cytoplasm of infected cells (i), whereas only few silver grains are present in uninfected cells (u). Starch (s) is visible as grayish grains in uninfected cells. K, Bright-field micrograph of section shown in J. Nuclei are stained blue. Bar = 10 µm.

The intensity of the signals obtained after hybridization with the PBG deaminase (Fig. 5, D and E) or with the coprogen oxidase (Fig. 5, G and H) probes was not as intense as with ALA dehydratase. However, the distribution of both PBG deaminase (Fig. 5D) and coprogen oxidase (Fig.5G) transcripts resembled that of ALA dehydratase, i.e. they were present in the central tissue, although there was no gradient of expression or an increased expression in the nodule inner cortex. No hybridization signal was obtained with sense probes for either of these two enzyme clones (Fig. 5, F and I).

In similar experiments on pea nodules, the results were the same. The transcripts of all three enzymes appeared even more distinct in infected cells of pea than in those of soybean because of the larger size of the pea nodule cells (Fig. 5, J and K). In both pea and soybean the signal intensities of the three enzyme transcripts were not significantly above background levels in root tissues adjacent to the nodule.

Leghemoglobin gene transcripts are located mainly in infected cells (de Billy et al., 1991; Tate et al., 1994). The plant heme synthesis enzymes are therefore increased in the same type of cells where the protein moiety of leghemoglobin is synthesized.

DISCUSSION

In this study we determined the expression of five heme synthesis enzymes during the nodulation of soybean and pea roots. All of these enzymes, ALA dehydratase, PBG deaminase, coprogen oxidase, protogen oxidase, and ferrochelatase, increase during nodulation, with a maximum at 3 weeks after infection with the appropriate inoculant (Fig. 4). The time courses of induction were virtually identical for each enzyme and paralleled the increase of both heme and holo-leghemoglobin in the nodule (Nadler and Avissar, 1977; Sangwan and O'Brian,

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1992). A similar observation was made for GSA aminotransferase, one of the enzymes of the C5 pathway (Sangwan and O'Brian, 1993), which was undetectable in uninfected roots, and increased to levels greater than in leaves 20 d after infection. This was paralleled by increases in transcript and enzyme protein (Frustaci et al., 1995). The same workers found that ALA dehydratase activity and message were high in nodules but were completely absent from uninfected roots (Kaczor et al., 1994). This differs somewhat from our results, in which ALA dehydratase was present before infection. Because the synthesis of heme is cell autonomous, the enzymes of the pathway would be expected to be present in all tissues of the plant to provide heme for cytochromes and other hemoproteins. The discrepancy might be explained by the fact that we used roots from 14-d-old plants, whereas Kaczor et al. (1994)measured activity in much older plants (23 d postinoculation). The detectable activity of ALA dehydratase and PBG deaminase in pea roots was found to be a maximum 5 d after germination and thereafter steadily declined (Smith, 1986). Additionally, maximum PBG deaminase activity was found in root tips compared with the older tissue in the elongation zone (Witty et al., 1993). Therefore, it is most likely that enzyme activity is present in roots but at levels too low to be assayed accurately. Similarly, we were unable to detect coprogen oxidase activity reproducibly in uninfected roots, most likely because of the limitations of the fluorimetric assay, which suffers from high background (Smith and Griffiths, 1993). In contrast, protein was detectable on western blots of uninfected roots (Fig. 3), and coprogen activity was measurable in roots (data not shown) using a more sensitive radiochemical assay (Elder and Evans, 1978).

Our assays of enzyme activity were carried out on extracts of whole, unfractionated nodules; therefore, it is not possible to distinguish whether the enzymes were plant or bacteria derived. However, support for the conclusion that it is the plant enzymes that are stimulated comes from the results of cell-fractionation studies. Sangwan and O'Brian (1991) found that ALA dehydratase and PBG deaminase levels were the same in free-living and symbiotic *B. japonicum*cells, and Jacobs et al. (1990) found increased protogen oxidase activity in the peribacteroid membrane (which is plant derived) compared with uninfected roots. We have obtained further evidence by investigating the levels of enzyme protein with antibodies and transcripts with the available cDNA probes. These molecular probes are specific for the plant enzymes and do not detect those from the symbiont. For coprogen oxidase, in situ hybridization confirmed earlier results (Madsen et al., 1993) that there is a specific increase in mRNA levels in nodules, and with western analysis we found a concomitant increase in enzyme protein, which correlates with the increase in enzyme activity. The similarly large increase in ALA dehydratase and PBG deaminase transcripts detected by northern analysis and in situ hybridization also provides evidence that the increase in enzyme activity is due to the plant-specific enzyme. Furthermore, the increased level of transcripts for the plant

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enzymes is specifically in the infected cells, where the apoprotein of leghemoglobin is exclusively synthesized (de Billy et al., 1991; Tate et al., 1994).

Thus, our results provide additional evidence for the involvement of the plant heme synthesis enzymes in the production of heme in the mature nodule. Because the major hemoprotein by far is leghemoglobin, the conclusion to be drawn is that the plant host provides some if not all of its prosthetic heme. At first this might seem contradictory to results obtained from the study of rhizobial mutants of heme synthesis. For instance, although *hemA* mutants (defective in ALA synthase) of *Rhizobium* sp. NGR234 (Stanley et al., 1988),*R. meliloti* (Leong et al., 1982, 1985; Mohapatra and Puhler, 1986; de Bruijn et al., 1989), and *Azorhizobium caulinodans*(Pawlowski et al., 1993) are able to elicit nodules on their corresponding host roots, the nodules are unable to fix nitrogen. Similarly, *B. japonicum* mutants defective in ALA dehydratase (*hemB*; Chauhan and O'Brian, 1993) and ferrochelatase (*hemH*; Frustaci and O'Brian, 1992) elicit such so-called Fix^- nodules. In all of these examples, there is no apoleghemoglobin. Another *B. japonicum* mutant lacking protogen oxidase activity, which also forms Fix^-nodules, nonetheless contains the leghemoglobin apoprotein (O'Brian et al., 1987) despite the lack of bacterial heme. In this case, the primary mutation has been demonstrated to be in a gene involved in the biosynthesis of *c*-type cytochromes (Ramseier et al., 1991). In contrast, a *hemA* mutant of *B. japonicum* MLG1, which has no detectable heme in the free-living form, induces the formation of functional nodules containing holo-leghemoglobin and other bacterial hemes (Guerinot and Chelm, 1986; Chauhan and O'Brian, 1993). This discrepancy has been explained by the proposal that ALA can be provided to the bacterial symbiont by the soybean host but that later intermediates cannot be transported across the peribacteroid membrane (Sangwan and O'Brian, 1991). Further evidence for this model comes from the observation that those *Rhizobium* species that require the *hemA* gene for symbiosis are severely deficient in ALA uptake activity (McGinnis and O'Brian, 1995).

Further work on the *hemA* mutants of *R. meliloti*using ultrastructural analysis, translation in vitro of plant RNA, and northern blots found that there was (a) atypical nodule morphology and infection thread development, (b) arrest early in development, and (c) only early nodulin genes expressed (Dickstein et al., 1991). Three of the mutants initiated nodules that did not contain any intracellular bacteria. A reduction in viable bacterial cells is observed in the*hemB* and *hemH* mutants as well (Frustaci and O'Brian, 1992; Chauhan and O'Brian, 1993). Thus, the Fix^- phenotype may not be a direct consequence of the bacteria's inability to supply heme for leghemoglobin but rather is a pleiotropic effect of the failure of nodule development.

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Although we have clearly demonstrated induction in the nodule of plant-specific enzymes of heme biosynthesis, it remains uncertain as to what signal leads to this induction. Although the time course of increase in the level of the coprogen oxidase transcript was the same as

that for leghemoglobin, there were no significant sequence similarities between the coprogen oxidase promoter and those of other late nodulin genes (Madsen et al., 1993). The induction is also unlikely to be in direct response to anoxia, which increases expression of the yeast coprogen oxidase gene (Zagorec and Labbe-Bois, 1986), because we could detect no increase in the soybean enzyme in roots subjected to anaerobiosis. This is perhaps not surprising, because although the nodule environment has a low free-oxygen content, this is mediated by leghemoglobin, which requires coprogen oxidase to provide its prosthetic group. Therefore, the requirement for increased coprogen oxidase activity would be before the production of a hypoxic environment. One interesting observation is that the coprogen oxidase gene appears to be induced in nodules to a much higher level than in leaves, even though the rates of tetrapyrrole synthesis are much greater in the latter (Table I). Similarly, protogen oxidase activity is greater in nodules than in leaves (Tables II and III). It is conceivable that the efficiency of extraction of the enzymes from the two tissues is different, although intuitively it would be expected to be easier from the softer leaf tissue. The present study is the first, to our knowledge, in which the activity of several of the tetrapyrrole synthesis enzymes has been determined in different tissues of the same plant, so this question must remain unresolved. Nonetheless, in nodules all of the enzymes are in vast excess over that required to sustain the observed rate of heme, even ALA dehydratase and PBG deaminase, which appear to be higher in leaves than in nodules.

An explanation for this apparent anomaly would be that there is considerable heme turnover; therefore, the rate we have calculated is an underestimate. Evidence for the ability of plants to turn over heme comes from the study of tetrapyrrole synthesis during the greening of etiolated seedlings, when there is massive flux through the chlorophyll branch of the pathway. When [^{14}C]ALA was administered to greening barley seedlings after 5 h of illumination, the specific radioactivities of extractable protoheme and pheophorbide (a chlorophyll derivative) were essentially the same, indicating that both were synthesized during the period. However, heme did not accumulate (Castelfranco and Jones, 1975). If a similar situation prevails in the nodule, the next obvious question is for what purpose is the heme synthesized? One possibility is that heme is not just required as a prosthetic group for leghemoglobin and respiratory cytochromes but that it is also involved in the regulation of nodule formation and/or function. Jensen et al. (1986) found that a heme-specific regulatory system in yeast modulated the translation of chimeric genes containing 5′ flanking sequences from the soybean leghemoglobin c_3 gene. More intriguingly, the*FixL* protein of *R. meliloti* has been shown to be a hemoprotein (Monson et al., 1992). *FixL* and*FixJ* form the two-component regulatory system involved in sensing and transducing the low-oxygen signal, which leads to expression of the rhizobial genes required for nitrogen fixation.

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In summary, the evidence presented in this paper supports that of earlier studies (Jacobs et al., 1990; Madsen et al., 1993; Sangwan and O'Brian, 1993; Kaczor et al., 1994, Frustaci et al., 1995; O'Brian, 1996), and together provide strong evidence that the plant cell is responsible for making at least some, if not all, of the heme moiety of leghemoglobin. Thus, the hypothesis that the plant host makes the apoprotein and the bacterial endosymbiont makes the prosthetic group (Appleby, 1984) is no longer tenable. Nonetheless, the fact that several rhizobial heme-synthesis mutants are unable to form functional nodules suggests the possibility that heme or some other tetrapyrrole is required as a signal for the induction of certain plant genes that are necessary for the later stages of nodule development and/or function.

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Abbreviations:

ALA 5-aminolevulinic acid

coprogen oxidase coproporphyrinogen III oxidase

GSA glutamate-1-semialdehyde

PBG porphobilinogen

protogen protoporphyrinogen IX

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Author notes

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Indu Sangwan et al., Plant Phyisol, 1999

Plant [delta]-Aminolevulinic Acid Dehydratase (Expression in Soybean Root Nodules and Evidence for a Bacterial Lineage of the Alad Gene)

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NLP1 reciprocally regulates nitrate inhibition of nodulation through SUNN-CRA2 signaling in Medicago truncatula

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U.S. Patent No. 10,039,306

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670 __ |**a** Work cat.: Acta Universitatis Lodziensis, 1986
906 __ |**t** 8888 |**u** tc00 |**v** 0
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321 Bimonthly, $b 1954-
321 Quarterly, $b 1926-1953
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362 1 Began with Jan. 1926 issue.
362 1 Ceased in print with: Vol. 166, no. 4 (Dec. 2014).
500 Publisher: American Society of Plant Biologists, <May 2001-Dec. 2014.
510 2 Chemical abstracts $x 0009-2258
580 Beginning with Aug. 1995 issue, Plant gene register section is no longer printed with the journal and is issued only online.
588 Description based on: Vol. 69, no. 1 (Jan. 1982); title from cover.
588 Latest issue consulted: Vol. 166, no. 4 (Dec. 2014).
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856 41 $u http://www.plantphysiol.org/
856 41 $u http://www.jstor.org/journals/00320889.html
856 42 $3 Plant gene register $u http://www.tarweed.com/

Attachment 46a

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150 __ |**a** Proteins

360 __ |**i** various protein groups and bodies, e.g. |**a** Fibrinogen, Peptones, Casein

450 __ |**a** Proteids

550 __ |**w** g |**a** Biomolecules

550 __ |**w** g |**a** Polypeptides

550 __ |**a** Proteomics

670 __ |**a** Oxford dict. of biochemistry and molecular biology, 2006 |**b** (protein: any of a large group of organic compounds found as major macromolecular constituents of living organisms. A protein is a linear polymer of amino acids linked by peptide bonds in a specific sequence. In the biosynthesis of the polypeptide chains, any of 20 different amino acids may be incorporated according to the genetic instructions of the cell.)

670 __ |**a** Facts on file dict. of biochemistry, 2003 |**b** (protein: one of a large number of substances that are important in the structure and function of all living organisms. Proteins are polypeptides; i.e., they are made up of amino acid molecules joined together by peptide links. Their molecular weight may vary from a few thousand to

several million. About 20 amino acids are present in proteins. Proteins may consist of one or several polypeptide chains.)

906 __ |**t** 0745 |**u** te04 |**v** 0

953 __ |**a** xx00 |**b** ta25

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150 __ |**a** Biochemistry

360 __ |**i** subdivision |**a** Composition |**i** under individual animals and groups of animals, e.g. |**a** Fishes--Composition; Cattle--Composition

450 __ |**w** nne |**a** Biological chemistry

450 __ |**a** Chemical composition of organisms

450 __ |**a** Organisms |**x** Composition

450 __ |**w** nne |**a** Physiological chemistry

550 __ |**w** g |**a** Biology

550 __ |**w** g |**a** Chemistry

550 __ |**w** g |**a** Medical sciences

670 __ |**a** MeSH browser, July 29, 2020 |**b** (Biochemistry. SN The study of the composition, chemical structures, and chemical reactions of living things. In MeSH Tree Structures under Biological Science Disciplines and under Chemistry)

670 __ |**a** Web. 3.

670 __ |**a** Hennepin.

670 __ |**a** McGraw-Hill dict. of sci. and tech. terms, c2003 |**b** (biochemistry [CHEM] The study of chemical substances occurring in living organisms and the reactions and methods for identifying these substances)

670 __ |**a** Tröjbom, M. Chemistry data from surface ecosystems in Forsmark and Laxemar-Simpevarp, 2010: |**b** p. 3 ("chemical composition of the regolith and biota of the terrestrial, limnic, and marine ecosystems")

670 __ |**a** Biology dictionary, via WWW, July 29, 2020 |**b** (Chemical Biology: a scientific discipline that combines chemistry and biology by using chemistry and chemical techniques to study biological systems. The main difference between chemical biology and biochemistry is that chemical biology involves adding novel chemical compounds to a biological system, while biochemistry is the study of chemical reactions that naturally happen inside organisms. Examples of chemical biology research include controlling cell division and other cell activities, using small molecules as targets for treatment, and manipulating stem cells. Differences between chemical biology and biochemistry: Chemical biology adds chemical compounds to biological systems in order to see what effect these chemicals have on cells and tissues. Chemical biologists often synthesize and add new compounds to cells. The goal of chemical biology research is to develop techniques that can eventually be applied to cells in a living organism, such as treatment options for cancer and other diseases. Biochemistry, on the other hand, is all about the chemical processes that already occur naturally in the cell. Instead of adding chemical compounds to cells to see the effects, biochemistry researchers study the chemical reactions that occur in organisms and the molecular makeup of these compounds. Biochemists also tend to study larger molecules like proteins and nucleic acids, while chemical biologists study smaller molecules) |**u** https://biologydictionary.net/chemical-biology/

680 __ |**i** Here are entered works on the study of naturally occurring chemical reactions in organisms. Works on the use of chemistry and chemical techniques to study biological systems are entered under |**a** Chemical biology.

681 __ |**i** Note under |**a** Chemical biology

910 __ |**a** Proposal saved by tc03 on 07/31/2020 at 10:12:44

952 __ |**a** Made for existing bibliographic records

952 __ |**a** LC pattern: Biophysics

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906 __ |**t** 2009 |**v** 0

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URL: https://catalog.loc.gov/

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Attachment 46d

OCLC 908639898 **No holdings in XXX - 35 other holdings**

Books

								Rec Stat		Entered	20150508	Replaced	20220419123603.4
Type	a	ELvl	l	Srce	d	Audn		Ctrl		Lang	eng
BLvl	m	Form		Conf	0	Biog		MRec		Ctry	mau
		Cont	b	GPub		LitF	0	Indx	1		
Desc	i	Ills	a	Fest	0	DtSt	t	Dates	2015, 2015		

040 XFF $b eng $e rda $c XFF $d XFF $d BTCTA $d YDXCP $d CRU $d CDX $d GZE $d OCLCF $d OCLCQ $d BDX $d UKMGB $d OCLCO
015 GBB512001 $2 bnb
016 7 017035973 $2 Uk
019 898052468 $a 898162403 $a 912238449
020 9780128028285
020 0128028289
020 $z 9780128028704 $q (PDF ebook)
050 4 RS431.P37 $b .D384 2015
050 4 QD431 $b .A3 v.98
082 04 615.6 $2 23
090 $b
245 00 Protein and peptide nanoparticles for drug delivery / $c edited by Rossen Donev.
264 1 Waltham, MA : $b Academic Press, $c [2015]
264 4 $c ©2015
300 xv, 369 pages : $b illustrations ; $c 24 cm.
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
490 1 Advances in protein chemistry and structural biology ; $v volume 98
504 Includes bibliographical references and indexes.
588 0 Online resource; title from title details screen (ScienceDirect, viewed April 2, 2015).
540 Current copyright fee: GBP19.00 $c 42\0. $5 Uk
650 0 Nanoparticles.
650 0 Peptide drugs $x Dosage forms.
650 0 Protein drugs $x Dosage forms.
650 2 Nanoparticles $0 (DNLM)D053758
650 6 Nanoparticules. $0 (CaQQLa)201-0262530
650 6 Médicaments peptidiques $x Formes pharmaceutiques. $0 (CaQQLa)201-0263775
650 6 Médicaments protéiques $x Formes pharmaceutiques. $0 (CaQQLa)201-0263778
650 7 Nanoparticles. $2 fast $0 (OCoLC)fst01032624
650 7 Peptide drugs $x Dosage forms. $2 fast $0 (OCoLC)fst01057557
650 7 Protein drugs $x Dosage forms. $2 fast $0 (OCoLC)fst01079681
700 1 Donev, Rossen, $e editor.
776 08 $i ebook version: $z 9780128028704
830 0 Advances in protein chemistry and structural biology ; $v volume 98.
856 $3 1850-9999 $u http://www.sciencedirect.com/ $x BLDSS

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Previous Next

MARC Display | Labelled Display

LC control no.: sh 85089689

LCCN Permalink: https://lccn.loc.gov/sh85089689

HEADING: Nanoparticles

000 02840cz a2200349n 450

001 4739952

005 20210112060037.0

008 860211i| anannbabn |a ana

010 __ |**a** sh 85089689

035 __ |**a** (DLC)sh 85089689

035 __ |**a** (DLC)6607048

035 __ |**a** (DLC)sp 85089689

035 __ |**a** (DLC)332293

040 __ |**a** DLC |**c** DLC |**d** DLC |**d** WaU |**d** DLC

053 _0 |**a** RS201.N35 |**c** Pharmacy

150 __ |**a** Nanoparticles

450 __ |**a** Nano-particles

450 __ |**a** NPs (Nanoparticles)

450 __ |**a** Nanoscale particles

550 __ |**w** g |**a** Nanostructured materials

550 __ |**w** g |**a** Particles

670 __ |**a** Wikipedia, Aug. 8, 2005 |**b** ("a nanoparticle is a microscopic particle whose size is measured in nanometers. Often such particles in nanoscale are used in biomedical applications acting as drug carriers or imaging agents.")

670 __ |**a** Britannica online, July 17, 2020 |**b** (Nanoparticle, ultrafine unit with dimensions measured in nanometres (nm; 1 nm = 10^{-9} metre). Nanoparticles exist in the natural world and are also created as a result of human activities. Because of their submicroscopic size, they have unique material characteristics, and manufactured nanoparticles may find practical applications in a variety of areas, including medicine, engineering, catalysis, and environmental remediation)

670 __ |**a** What are nanoparticles?, via TWI website, viewed July 17, 2020 |**b** (A nanoparticle is a small particle that ranges between 1 to 100 nanometres in size. Undetectable by the human eye, nanoparticles can exhibit significantly different physical and chemical properties to their larger material counterparts. The definition

given by the European Commission states that the particle size of at least half of the particles in the number size distribution must measure 100 nm or below. Most nanoparticles are made up of only a few hundred atoms) |**u** https://www.twi-global.com/technical-knowledge/faqs/what-are-nanoparticles

670 __ |**a** Journal of colloid and interface science, v. 521 (July 2018): |**b** p. 69 (article title: Nano-particle dynamics during capillary suction; nanoparticles)

670 __ |**a** Journal of environmental management, v. 196 (1 July 2017): |**b** p. 297 (article title: Engineered nano particles; abstr.: a wide variety of nano particles (NPs) are ubiquitously dispersed in ecosystems)

670 __ |**a** Springer handbook of nanotechnology, 2017: |**b** p. 643 (article title: Nano-particles for biomedical applications; Nanoparticles (NPs) are extremely small particulates with an average size that ranges from a micron or less to a few nanometers. The large majority of NPs necessitate nanotechnology methods for their production)

906 __ |**t** 0535 |**u** te04 |**v** 0

910 __ |**a** Record saved by jr02 on 01/11/2021 at 12:06:22

910 __ |**a** Proposal saved by tc03 on 07/22/2020 at 15:11:50

953 __ |**a** xx00 |**b** ta25

906 __ |**t** 2009 |**v** 0

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Attachment 46f

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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MARC Display | Labelled Display

LC control no.: sh 94005144
LCCN Permalink: https://lccn.loc.gov/sh94005144
HEADING: Peptide drugs Dosage forms

000 00565cz a2200193n 450
001 4860985
005 20120330081706.0
008 940711i| anannbabn |a ana
010 __ |**a** sh 94005144
035 __ |**a** (DLC)sh 94005144
035 __ |**a** (DLC)207670
040 __ |**a** DLC |**c** DLC
150 __ |**a** Peptide drugs |**x** Dosage forms
450 __ |**a** Dosage forms of peptide drugs
550 __ |**w** g |**a** Drug delivery systems
670 __ |**a** Work cat.: 94-3789: Formulation and delivery of proteins and peptides, 1994.
906 __ |**t** 9430 |**u** te04 |**v** 1
952 __ |**a** 0 bib. record(s) to be changed
953 __ |**a** jc02

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Attachment 46g

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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MARC Display | Labelled Display

LC control no.: sh 94005142
LCCN Permalink: https://lccn.loc.gov/sh94005142
HEADING: Protein drugs Dosage forms

000 00613cz a2200205n 450
001 4860983
005 20120330081705.0
008 940711i| anannbabn |a ana
010 __ |**a** sh 94005142
035 __ |**a** (DLC)sh 94005142
035 __ |**a** (DLC)207668
040 __ |**a** DLC |**c** DLC
150 __ |**a** Protein drugs |**x** Dosage forms
450 __ |**a** Dosage forms of protein drugs
550 __ |**w** g |**a** Drug delivery systems
670 __ |**a** Work cat.: 94-3789: Formulation and delivery of proteins and peptides, 1994.
906 __ |**t** 9430 |**u** te04 |**v** 1
952 __ |**a** 0 bib. record(s) to be changed
952 __ |**a** LC pattern: Drugs--Dosage forms
953 __ |**a** jc02

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Attachment 47a

001 992670000000062249
005 20200520144314.0
006 m o d |
007 cr -n---------
008 110202s2011 enkad ob 001 0 eng d
020 ##$a1-282-95479-2
020 ##$a9786612954795
020 ##$a0-12-380887-1
035 ##$a(CKB)2670000000062249
035 ##$a(EBL)625340
035 ##$a(OCoLC)700701869
035 ##$a(SSID)ssj0000469234
035 ##$a(PQKBManifestationID)12174053
035 ##$a(PQKBTitleCode)TC0000469234
035 ##$a(PQKBWorkID)10510476
035 ##$a(PQKB)11721065
035 ##$a(MiAaPQ)EBC625340
035 ##$a(Au-PeEL)EBL625340
035 ##$a(CaPaEBR)ebr10440521
035 ##$a(CaONFJC)MIL295479
040 ##$aMiAaPQ $cMiAaPQ $dMiAaPQ
041 ##$aeng
050 #4$aTX769 $b.F56 2011
082 00$a613.2 $a664.72272
245 00$aFlour and breads and their fortification in health and disease prevention $h[electronic resource] / $cedited by Victor R. Preedy, Ronald Ross Watson, Vinood B. Patel.
250 ##$a1st ed.
260 ##$aLondon : $bElsevier Science, $c2011.
300 ##$a1 online resource (543 p.)
336 ##$atext $btxt
337 ##$acomputer $bc
338 ##$aonline resource $bcr
500 ##$aDescription based upon print version of record.
520 ##$a Bread and flour-based foods are an important part of the diet for millions of people worldwide. Their complex nature provides energy, protein, minerals and many other macro- and micronutrients. However, consideration must be taken of three major aspects related to flour and bread. The first is that not all cultures consume bread made from wheat flour. There are literally dozens of flour types, each with their distinctive heritage, cultural roles and nutritive contents. Second, not all flours are used to make leavened bread in the traditional (i.e., Western) loaf form. There are many differe
546 ##$aEnglish
504 ##$aIncludes bibliographical references and index.
505 0#$asection 1. Flour and breads -- section 2. Fortification of flour and breads and their metabolic effects.
650 #0$aFlour $xAdditives.

```
650    #0$aFlour $xHealth aspects.
650    #0$aBread $xAdditives.
655    #4$aElectronic books.
776    ##$z0-12-380886-3
700    1#$aPreedy, Victor R.
700    1#$aWatson, Ronald R. $q(Ronald Ross)
700    1#$aPatel, Vinood B.
906    ##$aBOOK
```

Attachment 47b

OCLC 700701869 **No holdings in XXX - 506 other holdings**

Books

								Rec Stat		Entered	20110205	Replaced	20220726034231.4
Type	a	ELvl	M	Srce	d	Audn		Ctrl		Lang	eng		
BLvl	m	Form	o	Conf	0	Biog		MRec		Ctry	vtu		
		Cont		GPub		LitF	0	Indx	0				
Desc	u	Ills		Fest	0	DtSt	s	Dates	2011,				

007 c $b r $e n $g ---
040 MERUC $b eng $e pn $c MERUC $d EBLCP $d OCLCQ $d IDEBK $d OCLCQ $d UKDOC $d OCLCQ $d DEBSZ $d OCLCQ $d Z5A $d OCLCO $d ZCU $d OCLCQ $d MERUC $d OCLCQ $d OCLCO $d U3W $d OCLCF $d ICG $d AU@ $d OCLCO $d OCLCQ $d OCLCO $d OCLCA $d DKC $d OCLCQ $d OCLCO $d OCL
020 9780123808875
020 0123808871
050 4 TX558.W5
060 4 2011 C-160
060 4 QU 145.5
082 04 664.72272
090 $b
100 1 Preedy, Victor.
245 10 Flour and Breads and their Fortification in Health and Disease Prevention.
260 Burlington : $b Elsevier Science, $c 2011.
300 1 online resource (543 pages)
336 text $b txt $2 rdacontent
337 computer $b c $2 rdamedia
338 online resource $b cr $2 rdacarrier
505 0 Front Cover; Flour and Breads and their Fortification in Health and Disease Prevention; Copyright; Contents; LIST OF CONTRIBUTORS; PREFACE; Section 1 -Flour and Breads; Section 2 -Fortification of Flour and Breads and their Metabolic Effects; INDEX.
520 Bread and flour-based foods are an important part of the diet for millions of people worldwide. Their complex nature provides energy, protein, minerals and many other macro- and micronutrients. However, consideration must be taken of three major aspects related to flour and bread. The first is that not all cultures consume bread made from wheat flour. There are literally dozens of flour types, each with their distinctive heritage, cultural roles and nutritive contents. Secondly, not all flours are used to make leavened bread in the traditional (i.e., Western) loaf form.
588 0 Print version record.
650 0 Flour $x Additives.
650 0 Flour $x Health aspects.
650 0 Bread $x Additives.
650 0 Bread $x Health aspects.
650 0 Medicine, Preventive.
650 0 Enriched foods.
650 22 Food, Fortified $0 (DNLM)D005527
650 22 Nutritive Value $0 (DNLM)D009753
650 2 Preventive Medicine $0 (DNLM)D011315

650 6 Farine $0 (CaQQLa)201-0004136 $x Adjuvants. $0 (CaQQLa)201-0373850
650 6 Pain $0 (CaQQLa)201-0020898 $x Adjuvants. $0 (CaQQLa)201-0373850
650 6 Médecine préventive. $0 (CaQQLa)201-0001925
650 6 Aliments enrichis. $0 (CaQQLa)201-0062846
650 6 Valeur nutritive. $0 (CaQQLa)201-0198561
650 7 TECHNOLOGY & ENGINEERING $x Food Science. $2 bisacsh
650 7 Enriched foods. $2 fast $0 (OCoLC)fst00912556
650 7 Medicine, Preventive. $2 fast $0 (OCoLC)fst01015324
655 4 Electronic books.
700 1 Watson, Ronald Ross.
700 1 Patel, Vinood B.
776 08 $i Print version: $a Preedy, Victor. $t Flour and Breads and their Fortification in Health and Disease Prevention. $d Burlington : Elsevier Science, ©2011 $z 9780123808868
856 40 $3 123Library $u http://www.123library.org/book_details/?id=46263
856 40 $3 MyiLibrary $u http://www.myilibrary.com?id=295479
856 40 $3 ProQuest Ebook Central $u https://public.ebookcentral.proquest.com/choice/publicfullrecord.aspx?p=625340
856 41 $3 MyiLibrary, Table of contents $u http://www.myilibrary.com?id=295479&ref=toc

Attachment 47c

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

The Library's Main Reading Room will close to researchers earlier than usual, at 5:00pm, on Thurs., Nov. 3. All other reading rooms will be open according to their daily schedules.

LC control no.: sh 85049267
LCCN Permalink: https://lccn.loc.gov/sh85049267
HEADING: Flour

000 01076cz a2200289n 450
001 4700948
005 20211113060007.0
008 860211|| anannbabn |a ana
010 __ |**a** sh 85049267
035 __ |**a** (DLC)sh 85049267
035 __ |**a** (DLC)47633
040 __ |**a** DLC |**c** DLC |**d** DLC
053 _0 |**a** TS2120 |**b** TS2159 |**c** Manufacture
053 _0 |**a** TX393 |**c** Food supply
053 _0 |**a** TX558.W5 |**c** Food values
053 _0 |**a** TX595.F6 |**c** Adulteration
150 __ |**a** Flour
450 __ |**a** Flours
450 __ |**a** Wheat flour
550 __ |**w** g |**a** Plant products
670 __ |**a** Merriam-Webster dictionary, viewed online Sept. 3, 2021 |**b** (flour: noun, 1. a product consisting of finely milled wheat; also : a similar product made from another grain or food product (such as dried potatoes or fish) 2 : a fine soft powder)
906 __ |**t** 8824 |**u** fk05 |**v** 0
910 __ |**a** Record saved by jr02 on 11/12/2021 at 14:26:39
910 __ |**a** Record saved by jr02 on 09/03/2021 at 08:31:26
910 __ |**a** Record saved by jr02 on 09/03/2021 at 08:22:09
910 __ |**a** Record saved by jr02 on 09/03/2021 at 08:21:55
953 __ |**a** xx00 |**b** ff05

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Attachment 47d

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MARC Display | Labelled Display

LC control no.: sh 85000820

LCCN Permalink: https://lccn.loc.gov/sh85000820

HEADING: Additives

000 00445cz a2200157n 450

001 4654140

005 20110729100611.0

008 860211n| bnannbbbn |n ann

010 __ |**a** sh 85000820

035 __ |**a** (DLC)sh 85000820

035 __ |**a** (DLC)825

040 __ |**a** DLC |**c** DLC |**d** DLC

150 __ |**a** Additives

260 __ |**i** subdivision |**a** Additives |**i** under individual materials and types of materials, e.g. |**a** Concrete--Additives

906 __ |**t** 8836 |**u** fk05 |**v** 0

953 __ |**a** xx00 |**b** ta25

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Attachment 47e

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LC control no.: sh 85016646
LCCN Permalink: https://lccn.loc.gov/sh85016646
HEADING: Bread

000 00487cz a2200205n 450
001 4669350
005 20120322075746.0
008 860211i| anannbabn |a ana
010 __ |**a** sh 85016646
035 __ |**a** (DLC)sh 85016646
035 __ |**a** (DLC)16035
040 __ |**a** DLC |**c** DLC |**d** DLC
053 _0 |**a** TX769 |**c** Bread making
150 __ |**a** Bread
450 __ |**a** Breads
550 __ |**w** g |**a** Baked products
550 __ |**a** Cooking (Bread)
681 __ |**i** Example under |**a** Food
906 __ |**t** 9910 |**u** te07 |**v** 0
953 __ |**a** xx00 |**b** td04

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Attachment 47f

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

The Library's Main Reading Room will close to researchers earlier than usual, at 5:00pm, on Thurs., Nov. 3. All other reading rooms will be open according to their daily schedules.

LC control no.: sh 85083162

LCCN Permalink: https://lccn.loc.gov/sh85083162

HEADING: Medicine, Preventive

000 01151cz a2200313n 450

001 4733664

005 20120327093529.0

008 860211i| anannbabn |b ana

010 __ |**a** sh 85083162

035 __ |**a** (DLC)sh 85083162

035 __ |**a** (DLC)5805217

035 __ |**a** (DLC)sp 85083162

035 __ |**a** (DLC)293005

040 __ |**a** DLC |**c** DLC |**d** DLC |**d** InU |**d** DLC

053 _0 |**a** RA421 |**b** RA790

150 __ |**a** Medicine, Preventive

360 __ |**i** subdivision |**a** Prevention |**i** under individual diseases and types of diseases, e.g. |**a** Cancer--Prevention; |**i** and subdivision |**a** Diseases--Prevention |**i** under organs and regions of the body, e.g. |**a** Heart--Diseases--Prevention

450 __ |**a** Disease prevention

450 __ |**a** Diseases |**x** Prevention

450 __ |**a** Prevention of disease

450 __ |**a** Preventive medicine

550 __ |**a** Pathology

550 __ |**a** Preventive health services

550 __ |**a** Preventive medicine physicians

550 __ |**a** Public health

670 __ |**a** LC database, June 2, 1998 |**b** (disease prevention)

670 __ |**a** Report of the 1980 Invitational Conference on Disease Prevention and Health Promotion in Communities, 1980.

906 __ |**t** 0251 |**u** te04 |**v** 0

953 __ |**a** xx00 |**b** ta25

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Attachment 47g

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LC control no.: sh 85050240
LCCN Permalink: https://lccn.loc.gov/sh85050240
HEADING: Enriched foods

000 00503cz a2200205n 450
001 4701906
005 20120323073954.0
008 860211i| anannbabn |a ana
010 __ |**a** sh 85050240
035 __ |**a** (DLC)sh 85050240
035 __ |**a** (DLC)48591
040 __ |**a** DLC |**c** DLC |**d** DLC
150 __ |**a** Enriched foods
450 __ |**w** nne |**a** Food, Enriched
450 __ |**a** Fortified foods
550 __ |**w** g |**a** Food
550 __ |**a** Food additives
906 __ |**t** 8847 |**u** fk07 |**v** 0
952 __ |**a** 7 bib. records to be changed
953 __ |**a** xx00 |**b** fj21

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Library of Congress Online Catalog
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Attachment 48a

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005    20160204151409.0
008    050223s2005    caua   f b    001 0 eng
015    ##$aGBA525009 $2bnb
016    7#$a013146928 $2Uk
020    ##$a0123116325
020    ##$a9780123116321
035    ##$a(OCoLC)58828180
035    ##$a(MoKL)886504-lhalldb
035    ##$a(lhalldb)886504-lhalldb
040    ##$aUKM $beng $cUKM $dBAKER $dWAU $dIXA $dNLGGC $dAGL $dYDXCP
$dBTCTA $dLVB $dAU@ $dINCSK $dZVM $dBDX $dOCLCF $dOCLCO $dOCLCQ
$dLHL
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100    1#$aHan, Jung H.
245    10$aInnovations in food packaging / $cedited by Jung H. Han.
260    ##$aSan Diego, Calif. : $bElsevier Academic ; $aOxford :
$bElsevier Science [distributor], $c©2005.
263    ##$a2005
300    ##$axiii, 517 pages : $billustrations (some color) ; $c25 cm.
336    ##$atext $btxt $2rdacontent
337    ##$aunmediated $bn $2rdamedia
338    ##$avolume $bnc $2rdacarrier
490    1#$aFood science and technology international series
504    ##$aIncludes bibliographical references and index.
590    ##$aDonated by Sosland.
650    #0$aFood $xPackaging.
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700    1#$aHan, Jung H.
830    #0$aFood science and technology international series.
948    ##$aLTI 02/04/2016
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Attachment 48b

OCLC 58828180 **No holdings in XXX - 119 other holdings**

Books

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084 58.34 $2 bcl
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100 1 Han, Jung H.
245 10 Innovations in food packaging / $c edited by Jung H. Han.
260 San Diego, Calif. : $b Elsevier Academic ; $a Oxford : $b Elsevier Science [distributor], $c ©2005.
300 xiii, 517 pages : $b illustrations (some color) ; $c 25 cm.
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
490 1 Food science and technology international series
504 Includes bibliographical references and index.
520 Packaging performs a number of functions in the containment, protection, shipment and selling of goods. Innovations in Food Packaging recognizes that food packaging is a fast-growing area that impacts upon the important areas of product shelf-life and food safety. Each chapter provides information on the scientific background, new material development and utilization, and case studies of the use of new system for perishable food products. Covers four major food packaging topics: * Theories in food packaging * Active packaging * Modified atmosphere packaging * Edible films and coatings.
505 00 $g 1. $t New technologies in food packaging : overview / $r Jung H. Han -- $g 2. $t Mass transfer of gas and solute through packaging materials / $r Jung H. Han and Martin G. Scanlon -- $g 3. $t Quality of packaged foods / $r Rakesh K. Singh and Nepal Singh -- $g 4. $t Surface chemistry of food, packaging and biopolymer materials / $r Jung H. Han, Yachuan Zhang and Roberto A. Buffo -- $g 5. $t Introduction to active food packaging technologies / $r Michael L. Rooney -- $g 6. $t Antimicrobial packaging systems

/ $r Jung H. Han -- $g 7. $t Packaging containing natural antimicrobial or antioxidative agents / $r Dong Sun Lee -- $g 8. $t Oxygen-scavenging packaging / $r Michael L. Rooney -- $g 9. $t Intelligent packaging / $r Jung H. Han, Colin H. L. Ho and Evangelina T. Rodrigues -- $g 10. $t Introduction to modified atmosphere packaging / $r John D. Floros and Konstantinos I. Matsos -- $g 11. $t Internal modified atmospheres of coated fresh fruits and vegetables : understanding relative humidity effects / $r Luis Cisneros-Zevallos and John M. Krochta -- $g 12. $t Modified atmosphere packaging of ready-to-eat foods / $r Kevin C. Spencer -- $g 13. $t Preservative packaging for fresh meats, poultry, and fin fish / $r Alexander O. Gill and Colin O. Gill -- $g 14. $t Centralized packaging systems for meats / $r Roberto A. Buffo and Richard A. Holley -- $g 15. $t Edible films and coatings : a review / $r Jung H. Han and Aristippos Gennadios -- $g 16. $t Agro-polymers for edible and biodegradable films : review of agricultural polymeric materials, physical and mechanical characteristics / $r Stephane Guilbert and Nathalie Gontard -- $g 17. $t Edible films and coatings from plant origin proteins / $r Roberto A. Buffo and Jung H. Han -- $g 18. $t Edible films and coatings from animal-origin proteins / $r Monique Lacroix and Kay Cooksey -- $g 19. $t Edible films and coatings from starches / $r Zhiqiang Liu -- $g 20. $t Edible films and coatings from non-starch polysaccharides / $r Monique Lacroix and Canh Le Tien -- $g 21. $t Lipid-based edible films and coatings / $r Jong Whan Rhim and Thomas H. Shellhammer -- $g 22. $t Emulsion and bi-layer edible films / $r Maria B. Perez-Gago and John M. Krochta -- $g 23. $t Plasticizers in edible films and coatings / $r Rungsinee Sothornvit and John M. Krochta -- $g 24. $t Sensory quality of foods associated with edible film and coating systems and shelf-life extension / $r Yanyun Zhao and Mina McDaniel -- $g 25. $t Commercial uses of active food packaging and modified atmosphere packaging systems / $r Aaron L. Brody -- $g 26. $t US Food and Drug Administration approach to regulating intelligent and active packaging components / $r Yoon S. Song and Mark A. Hepp -- $g 27. $t Packaging for non-thermal food processing / $r Seacheol Min and Q. Howard Zhang.
650 0 Food $x Packaging.
650 0 Food $x Packaging $x Technological innovations.
650 6 Aliments $x Conditionnement. $0 (CaQQLa)201-0043064
650 6 Aliments $0 (CaQQLa)201-0043064 $x Conditionnement $0 (CaQQLa)201-0043064 $x Innovations. $0 (CaQQLa)201-0379286
650 7 Food $x Packaging. $2 fast $0 (OCoLC)fst00930550
650 7 Food $x Packaging $x Technological innovations. $2 fast $0 (OCoLC)fst00930561
650 17 Verpakking. $2 gtt
650 17 Levensmiddelen. $2 gtt
653 00 voedselverpakking
653 00 food packaging
653 10 Food Packaging
653 10 Verpakking van levensmiddelen
655 4 Electronic books.
700 1 Han, Jung H.
830 0 Food science and technology international series.

Attachment 48c

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HEADING: Food Packaging

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450 __ |**a** Groceries |**x** Packaging
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Attachment 49a

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082 0#$a664 $223
100 1#$aFellows, P. J.
245 10$aFood processing technology : $bprinciples and practice / $cP. J. Fellows.
250 ##$aThird edition.
264 #1$aCambridge, England ; $aBoca Raton, Florida : $bWoodhead Publishing : $bCRC Press, $c2009.
264 #4$c©2009
300 ##$a1 online resource (932 p.)
336 ##$atext $btxt
337 ##$acomputer $bc
338 ##$aonline resource $bcr
440 #0$aWoodhead Publishing in food science, technology, and nutrition
505 0#$aCover; Food processing technology:Principles and practice; Copyright; Contents; Acknowledgements; Dedication; Introduction; A brief history of food processing; The food industry today; About this book; References; Part I Basic principles; 1 Properties of food and processing; 1.1 Properties of foods; 1.2 Food spoilage, safety and shelf-life; 1.3 Types of processing; 1.4 Effects of processing; 1.5 Control of processing; References; Part II Ambient-temperature processing; 2 Raw material preparation; 2.1 Cooling crops and carcasses; 2.2 Cleaning; 2.3 Sorting and grading; 2.4 Peeling; References
505 8#$a3 Size reduction 3.1 Size reduction of solid foods; 3.2 Size reduction in liquid foods; References; 4 Mixing and forming; 4.1 Mixing; 4.2 Forming; References; 5 Separation and concentration of food components; 5.1 Centrifugation; 5.2 Filtration; 5.3

Expression; 5.4 Extraction using solvents; 5.5 Membrane concentration; References; 6 Food biotechnology; 6.1 Genetic modification; 6.2 Nutritional genomics; 6.3 Functional foods; 6.4 Fermentation technology; 6.5 Microbial enzymes; 6.6 Microencapsulation and controlled release technologies; 6.7 Bacteriocins and antimicrobial ingredients; References
505 8#$a7 Irradiation 7.1 Theory; 7.2 Equipment; 7.3 Applications; 7.4 Effect on Foods; 7.5 Effect on micro-organisms; 7.6 Effect on packaging; 7.7 Detection of irradiated foods; References; 8 High-pressure processing; 8.1 Theory; 8.2 Equipment; 8.3 Effect on parasites and micro-organisms; 8.4 Effect on enzymes; 8.5 Effects on foods; 8.6 Combinations of high pressure and other minimal processing technologies; References; 9 Minimal processing methods under development; 9.1 Pulsed electric field processing; 9.2 Processing using electric arc discharges; 9.3 Processing using oscillating magnetic fields
505 8#$a9.4 Processing using pulsed light and UV light 9.5 Processing using pulsed X-rays; 9.6 Processing using ultrasound; References; Part III Processing by application of heat; 10 Heat processing; 10.1 Theory; 10.2 Sources of heat and methods of application to foods; 10.3 Effect of heat on micro-organisms and enzymes; 10.4 Effect of heat on nutritional and sensory characteristics of foods; References; III.A Heat processing using steam or water; 11 Blanching; 11.1 Theory; 11.2 Equipment; 11.3 Effect on foods; 11.4 Effect on micro-organisms; References; 12 Pasteurisation; 12.1 Theory; 12.2 Equipment
505 8#$a12.3 Effect on foods References; 13 Heat sterilisation; 13.1 In-container sterilisation; 13.2 Ultra-high-temperature (UHT)/aseptic processes; 13.3 Effect on foods; References; 14 Evaporation and distillation; 14.1 Evaporation; 14.2 Distillation; References; 15 Extrusion; 15.1 Extrusion cooking theory; 15.2 Equipment; 15.3 Applications; 15.4 Effect on foods and micro-organisms; References; III.B Heat processing using hot air; 16 Dehydration; 16.1 Theory; 16.2 Equipment; 16.3 Control of dryers; 16.4 Rehydration; 16.5 Effect on foods and micro-organisms; References; 17 Smoking; 17.1 Theory
505 8#$a17.2 Processing
520 ##$aThe first edition of Food processing technology was quickly adopted as the standard text by many food science and technology courses. This completely revised and updated third edition consolidates the position of this textbook as the best single-volume introduction to food manufacturing technologies available. This edition has been updated and extended to include the many developments that have taken place since the second edition was published. In particular, advances in microprocessor control of equipment, 'minimal' processing technologies, functional foods, developments in 'active'
546 ##$aEnglish
500 ##$a"Reprinted in 2008"--T. p. verso.

504 ##$aIncludes bibliographical references at the end of each chapters and index.
588 ##$aDescription based on online resource; title from PDF title page (ebrary, viewed February 8, 2014).
650 #0$aFood industry and trade.
655 #4$aElectronic books.
710 2#$aScienceDirect (Servicio en línea)
776 ##$z1-84569-216-0
830 #0$aWoodhead Publishing Series in Food Science, Technology and Nutrition
906 ##$aBOOK

Attachment 49b

OCLC 798341671 **No holdings in XXX - 467 other holdings**

Books

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072 7 TEC $2 eflch
082 04 664.02 $2 22
090 $b
100 1 Fellows, P. $q (Peter), $d 1953-
245 10 Food processing technology : $b Principles and practice / $c P.J. Fellows.
250 3rd rev. ed.
260 Cambridge : $b Woodhead Publishing Ltd, $c 2009.
300 1 online resource (928 pages).
336 text $b txt $2 rdacontent
337 computer $b c $2 rdamedia
338 online resource $b cr $2 rdacarrier
490 1 Woodhead Publishing series in food science, technology and nutrition ; $v 174
505 0 Part 1 Basic principles: Properties of foods. Part 2 Ambient-temperature processing: Raw material preparation-- Size reduction-- Mixing and forming-- Separation and concentration of food components-- Food biotechnology-- Irradiation-- High-pressure processing-- Minimal processing methods under development. Part 3 Processing by application of heat: Heat processing-- Heat processing using steam or water-- Pasteurisation-- Heat sterilisation-- Evaporation and distillation-- Extrusion-- Heat processing using hot air-- Smoking-- Baking and roasting-- Frying-- Heat processing by direct and radiated energy. Part 4 Processing by removal of heat: Chilling and modified atmospheres-- Freezing-- Freeze drying and freeze concentration. Part 5 Post-processing operations: Coating-- Packaging-- Filling and sealing of containers-- Materials handling and process control.
520 The first edition of Food processing technology was quickly adopted as the standard text by many food science and technology courses. This completely revised and updated third edition consolidates the position of this textbook as the best single-volume introduction to food manufacturing technologies available. $b ' ... a well-written and authoritative review of food processing technology - the essential reference for food technologists and students alike.' Food Trade Review (of a previous edition) 'Interesting, logical and concise. A good introduction for students of nutrition, food science and technology, catering or agriculture.' Food Manufacture (of a previous edition) Widely regarded as a

standard work in its field, this book introduces the range of processing techniques that are used in food manufacturing. It explains the principles of each process, the processing equipment used, operating conditions and the effects of processing on micro-organisms that contaminate foods, the biochemical properties of foods and their sensory and nutritional qualities. The third edition is divided into five parts. Part one describes some important basic concepts. Part two describes unit operations that take place at ambient temperature or involve minimum heating of foods. Part three includes operations that heat foods to preserve them or to alter their eating quality. Part four describes operations that remove heat from foods to extend their shelf life with minimal changes in nutritional quality or sensory characteristics. Part five describes post-processing operations, including packaging and distribution logistics. The third edition has been substantially rewritten, updated and extended to include the many developments in food technology that have taken place since the second edition was published in 2000. Nearly all unit operations have undergone significant developments, and these are reflected in the large amount of additional material in each chapter. In particular, advances in microprocessor control of equipment, 'minimal' processing technologies, genetic modification of foods, functional foods, developments in 'active' or 'intelligent' packaging, and storage and distribution logistics are described. Developments in technologies that relate to cost savings, environmental improvement or enhanced product quality are highlighted. Additionally, sections in each chapter on the impact of processing on food-borne micro-organisms are included for the first time. The third edition consolidates the position of Food processing technology as the best single-volume introduction to food manufacturing technologies. It is an essential text for undergraduate and postgraduate students in food technology, food science, food marketing and distribution, agriculture, nutrition, and hospitality management/catering. It is also an ideal reference for all professionals in the food industry.
545 0 Dr Peter Fellows is a senior consultant in food processing, working mostly in Africa and Asia. Over the last 30 years, he has worked extensively as a food technologist in over twenty countries, supporting institutions that promote small-scale agro-industrial development programmes. He is a Visiting Fellow at Oxford Brookes University in the UK, and has held the UNESCO Chair in Post-Harvest Technology at Makerere University, Uganda. In addition to Food processing technology, he has written 22 books and numerous articles on different aspects of food processing.
650 0 Food industry and trade.
650 7 Food industry and trade. $2 fast $0 (OCoLC)fst00930843
655 4 Electronic books.
776 08 $i Print version: $a Fellows, P J. $t Food Processing Technology : Principles and Practice. $d Burlington : Elsevier Science, ©2009 $z 9781845692162
830 0 Woodhead Publishing in food science, technology, and nutrition ; $v 174.
856 40 $3 Ebook Library $u http://public.eblib.com/choice/publicfullrecord.aspx?p=1639821
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856 4 $3 Ebook Library $u http://public.eblib.com/choice/publicfullrecord.aspx?p=1639821

Attachment 50a

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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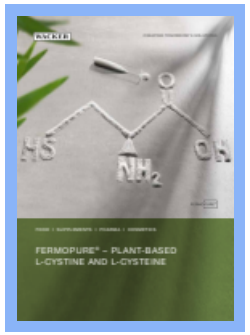

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Attachment 51a

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

Cambridge Core

Access

English | **Français**

Public health implications of meat production and consumption

Published online by Cambridge University Press: **02 January 2007**

Polly Walker, Pamela Rhubart-Berg, Shawn McKenzie, Kristin Kelling and Robert S Lawrence

Article contents

Abstract

HTML view is not available for this content. However, as you have access to this content, a full PDF is available via the 'Save PDF' action button.

The high level of meat and saturated fat consumption in the USA and other high-income countries exceeds nutritional needs and contributes to high rates of chronic diseases such as cardiovascular disease, diabetes mellitus and some cancers. Affluent citizens in middle- and low-income countries are adopting similar high-meat diets and experiencing increased rates of these same chronic diseases. The industrial agricultural system, now the predominant form of agriculture in the USA and increasingly world-wide, has consequences for public health owing to its extensive use of fertilisers and pesticides, unsustainable use of resources and environmental pollution. In industrial animal production there are public health concerns surrounding feed formulations that include animal tissues, arsenic and antibiotics as well as occupational health risks and risks for nearby communities. It is of paramount importance for public health professionals to become aware of and involved in how our food is produced.

Keywords

Motif Exhibit 1011, Page 843 of 1241

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

Diet Environment Health Nutrition Industrial agriculture Industrial animal production Sustainability Sustainable agriculture Aquaculture Meat

Type

Invited Paper

Information

Public Health Nutrition, Volume 8, Issue 4, June 2005, pp. 348 - 356
DOI: https://doi.org/10.1079/PHN2005727

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British Journal of Nutrition

Published online: 8 March 2007

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246	1_ | ‡**a** PHN
260	__ | ‡**a** Wallingford, Oxon, UK :‡**b** Published on behalf of the Nutrition Society by CAB International,‡**c** c1998-
260	3_ | ‡**3** <2007- > :‡**a** Oxford :‡**b** Cambridge University Press
300	__ | ‡**a** v. :‡**b** ill.
310	__ | ‡**a** Eighteen issues a year,‡**b** 2015-

321 __ | ‡**a** Quarterly,‡**b** -2001
321 __ | ‡**a** 6 times per year,‡**b** 2002
321 __ | ‡**a** 8 times per year,‡**b** 2003-2006
321 __ | ‡**a** Monthly,‡**b** 2007-2014
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337 __ | ‡**a** unmediated‡**b** n‡**2** rdamedia
338 __ | ‡**a** volume‡**b** nc‡**2** rdacarrier
362 0_ | ‡**a** Vol. 1, no. 1 (Mar. 1998)-
500 __ | ‡**a** Contents page and abstracts available on the Internet before publication.
500 __ | ‡**a** Title from cover.
510 1_ | ‡**9** 1‡**a** MEDLINE‡**b** v1n1, Mar. 1998-
510 0_ | ‡**9** 1‡**a** PubMed‡**b** v1n1, Mar. 1998-
510 1_ | ‡**a** Index medicus
510 0_ | ‡**a** PMC Forthcoming
530 __ | ‡**a** Also issued online.
583 __ | ‡**3** v.1, no. 1 (March 1998)‡**c** 19990728‡**f** Notice published‡**l** Printed on acid-free paper
650 02 | ‡**a** Diet
650 02 | ‡**a** Health Promotion
650 02 | ‡**a** Nutritional Physiological Phenomena
650 02 | ‡**a** Public Health
655 _2 | ‡**a** Periodical
710 2_ | ‡**a** Nutrition Society (Great Britain)
856 4_ | ‡**u** https://www.cambridge.org/core/journals/public-health-nutrition
911 __ | ‡**a** Electronic and print, print canceled 2018
914 __ | ‡**a** Portico‡**b** Current to 2022 as of 20220627‡**c** ISSN_13689800
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Attachment 51c

National Library of Medicine

Health Promotion MeSH Descriptor Data 2022

Details | Qualifiers | MeSH Tree Structures | Concepts

MeSH Heading	Health Promotion
Tree Number(s)	I02.233.332.445 N02.421.726.407.579
Unique ID	D006293
RDF Unique Identifier	http://id.nlm.nih.gov/mesh/D006293
Annotation	IM
Scope Note	Encouraging consumer behaviors most likely to optimize health potentials (physical and psychosocial) through health information, preventive programs, and access to medical care.
Entry Term(s)	Health Campaigns Promotion of Health Promotional Items Wellness Programs
See Also	Health Behavior
Public MeSH Note	80
History Note	80
Date Established	1980/01/01
Date of Entry	1979/06/01
Revision Date	2017/07/11

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Attachment 51d

National Library of Medicine

Public Health MeSH Descriptor Data 2022

Details | Qualifiers | MeSH Tree Structures | Concepts

MeSH Heading	Public Health
Tree Number(s)	H02.403.720 N01.400.550 N06.850
Unique ID	D011634
RDF Unique Identifier	http://id.nlm.nih.gov/mesh/D011634
Annotation	specialty; do not confuse with NATIONAL HEALTH PROGRAMS
Scope Note	Branch of medicine concerned with the prevention and control of disease and disability, and the promotion of physical and mental health of the population on the international, national, state, or municipal level.
Entry Term(s)	Community Health Environment, Preventive Medicine & Public Health Environment, Preventive Medicine and Public Health
See Also	Preventive Medicine Social Medicine Zoonoses
Entry Combination	organization & administration:Public Health Administration
Date Established	1966/01/01
Date of Entry	1999/01/01
Revision Date	2018/02/28

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Attachment 51e

OCLC 39319725 **No holdings in XXX - 90 other holdings**

Continuing Resources

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060 00 W1 $b PU535
070 0 RA784.P83
072 0 T000
072 0 X380
090 $b
210 10 Public Health Nutr $2 dnlm
245 00 Public health nutrition.
260 Wallingford, Oxon, UK : $b Published on behalf of the Nutrition Society by CAB International, $c ©1998-
260 3 $3 <2007- : $a Oxford : $b Cambridge University Press
300 volumes : $b illustrations ; $c 28 cm
310 Eighteen issues a year, $b 2015-
321 Quarterly, $b -2001
321 6 times per year, $b 2002
321 8 times per year, $b 2003-2006
321 Monthly, $b 2007-2014
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
362 0 Vol. 1, no. 1 (Mar. 1998)-
500 Contents page and abstracts available on the Internet before publication.
500 Title from cover.

500 Published by: Oxford :Cambridge University Press, <2007-
588 Latest issue consulted: Vol. 11, no. 3 (Mar. 2008).
650 0 Nutrition $v Periodicals.
650 0 Diseases $x Nutritional aspects $v Periodicals.
650 0 Public health $v Periodicals.
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650 0 Health promotion.
650 0 Nutrition.
650 0 Public health.
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653 PUBLIC HEALTH
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856 42 $z Scope $u http://firstsearch.oclc.org/journal=1368-9800;screen=info;ECOIP $z Address for accessing the journal from an authorized IP address through OCLC FirstSearch Electronic Collections Online

Attachment 51f

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

LC control no.: sh 85038411
LCCN Permalink: https://lccn.loc.gov/sh85038411
HEADING: Diseases

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008 860211i| anannbabn |a ana
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035 __ |**a** (DLC)sh 85038411
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040 __ |**a** DLC |**c** DLC |**d** DLC
150 __ |**a** Diseases
360 __ |**i** individual diseases, e.g. |**a** Cancer; |**i** and subdivision |**a** Diseases |**i** under individual organs and regions of the body, individual animals and groups of animals, classes of persons and ethnic groups, e.g. |**a** Heart--Diseases; Fishes--Diseases; Infants--Diseases; African Americans--Diseases
450 __ |**a** Human beings |**x** Diseases
450 __ |**a** Illness
450 __ |**a** Illnesses
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450 __ |**a** Sickness
450 __ |**a** Sicknesses
550 __ |**w** g |**a** Medicine
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550 __ |**a** Pathology
550 __ |**a** Sick
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953 __ |**a** xx00 |**b** te01

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Attachment 51g

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

LC control no.: sh2008110201
LCCN Permalink: https://lccn.loc.gov/sh2008110201
HEADING: Public health Periodicals

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667 __ |**a** Record generated for validation purposes.
670 __ |**a** Work cat.: Kyōto-shi Eisei Kōgai Kenkyūjo nenpō =, Heisei 3-nen [1991]-
906 __ |**t** 8888 |**u** te00 |**v** 0
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Attachment 51h

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

LC control no.: sh 85037854
LCCN Permalink: https://lccn.loc.gov/sh85037854
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450 __ |**a** Sick |**x** Nutrition
550 __ |**w** g |**a** Dietetics
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953 __ |**a** xx00 |**b** ta25

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Attachment 51i

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

LC control no.: sh 85059591
LCCN Permalink: https://lccn.loc.gov/sh85059591
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906 __ |**t** 9436 |**u** te03 |**v** 1
953 __ |**a** xx00 |**b** ta25

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Nutritional composition of red meat

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This section begins with the nutritional composition of red meat, and then focuses on the key nutrients delivered through the consumption of red meat in the context of the Australian diet. The reviews draw on the scientific literature to provide an overview of the metabolism and associations with clinical conditions of each of these nutrients. They then provide a perspective on the contributions of red meat in the diet to meet nutritional requirements. Williams provides up-to-date nutritional composition information; Truswell outlines the clinical conditions associated with vitamin B12 deficiency; Samman focuses on metabolism, food sources and requirements for iron and zinc; and Howe and colleagues provide an update on the nutritional implications of the long-chain omega-3 fatty acids. To conclude this section, Baghurst provides a perspective on food guides and the implications for red meat as a core food in the diet

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KEY POINTS

The lean component of red meat is:

- An excellent source of high biological value protein, vitamin B12, niacin, vitamin B6, iron, zinc and phosphorus
- A source of long-chain omega-3 polyunsaturated fats, riboflavin, pantothenic acid, selenium and, possibly, also vitamin D
- Relatively low in fat and sodium

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- A source of a range of endogenous antioxidants and other bioactive substances, including taurine, carnitine, carnosine, ubiquinone, glutathione and creatine

INTRODUCTION

The Food Standards Australia New Zealand (FSANZ) Food Standards Code defines meat as 'the whole or part of the carcass of any buffalo, camel, cattle, deer, goat, hare, pig, poultry, rabbit or sheep, slaughtered other than in a wild state, but does not include eggs, or foetuses'.[1] This definition does not include kangaroo meat, which is now widely available for purchase in Australia and is likely to be considered as meat.

The term 'meat' may refer only to meat flesh (skeletal muscle plus any attached connective tissue or fat), but the FSANZ definition also includes offal (i.e. meat other than meat flesh, including brain, heart, kidney, liver, pancreas, spleen, thymus, tongue and tripe) although it excludes bone and bone marrow.

In Australia, the term 'red meat' is used by the meat industry to refer to meat from cattle, sheep and goat (i.e. beef, veal, lamb, mutton and goat meat). It does not include meat from pigs (e.g. pork, bacon, ham) or kangaroo, nor less common game meats like buffalo and camel, although nutrient composition of some of these products is now becoming available.[2] Purchased red meat usually consists of both lean tissue (muscle) and fat tissue, which can be either distributed throughout the muscle as marbling (internal fat) or surrounding the muscle meat as selvage or external fat. In trimmed lean meat, it is usually the external fat only that is removed.[3] Processed meat means a product containing no less than 30% meat, that has undergone a method of preservation other than freezing, and includes manufactured meat and cured and/or dried meat flesh (e.g. sausages, salami, canned meats).[1] This paper outlines the key nutrients for which red meat could be considered a significant dietary source based on data of relative composition.

NUTRIENT COMPOSITION OF RED MEAT

Red meat contains high biological value protein and important micronutrients that are needed for good health throughout life. It also contains a range of fats, including essential omega-3 polyunsaturated fats. Recent analyses have shown that there has been a significant trend to leaner cuts of meat over the past two decades.[4] While the nutritional composition will vary somewhat according to breed, feeding regimen, season and meat cut, the following discussion will show that, in general, lean red meat has a relatively low fat content, is moderate in cholesterol, and is rich in protein and many essential vitamins and minerals.

Table 1 presents the typical nutrient composition of samples of fat-trimmed Australian red meat (beef, veal, lamb and mutton), based on recent analyses of national retail samples,[5-7] and compares this with the new Australian recommended dietary intakes (RDI).[8] While there are

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some differences between the four meats, in general lean red meat is a particularly good source of protein, niacin, vitamin B6, vitamin B12, phosphorus, zinc and iron, with 100 gproviding more than 25% RDI of these nutrients. It also provides more than 10% RDI of riboflavin, pantothenic acid and selenium. Of the four meats, mutton is particularly nutrient-dense, and the richest source of thiamin, vitamins B6 and B12, phosphorus, iron and copper.

Table 1. Average nutrient composition (per 100 g) of the lean component of Australian red meat[5-7]

	Beef [(a)]	*Veal* [(b)]	*Lamb* [(c)]	*Mutton* [(d)]	*Adult Australian RDI*
Moisture (g)	73.1	74.8	72.9	73.2	
Protein (g)	23.2	24.8	21.9	21.5	46–64
Fat (g)	2.8	1.5	4.7	4.0	–
Energy (kJ)	498	477	546	514	6.5–15.8 MJ
Cholesterol (mg)	50	51	66	66	–
Thiamin (mg)	0.04	0.06	0.12	0.16	1.1–1.2
Riboflavin (mg)	0.18	0.20	0.23	0.25	1.1–1.6
Niacin (mg)	5.0	16.0	5.2	8.0	14–16
Vitamin B6 (mg)	0.52	0.8	0.10	0.8	1.3–1.7
Vitamin B12 (μg)	2.5	1.6	0.96	2.8	2.4
Pantothenic acid (mg)	0.35	1.50	0.74	1.33	4–6
Vitamin A (μg)	<5	<5	8.6	7.8	700–900 μg RE[(e)]
Beta-carotene (μg)	10	<5	<5	<5	700–900 μg RE[(e)]
Alpha-tocopherol (mg)	0.63	0.50	0.44	0.20	7–10
Sodium (mg)	51	51	69	71	460–920

[(a)] Mean values for diced, stir-fry, round, rump, topside, silverside, fillet, sirloin, scotch fillet, T-bone, blade and chuck steak.

[(b)] Mean values for stir-fry, diced, leg steak and culet.

[(c)] Mean values for diced, stir-fry, leg roast, easy-carve roast, mini-roast, chump chop, loin chop, cutlet and easy-carve shoulder.

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(d) Mean values for leg roast and casserole mutton.

(e) RE = retinol equivalents (=1 μg retinol or 6 μg or beta-carotene).

RDI = recommended dietary intake.

Protein and amino acids

Raw red muscle meat contains around 20–25 g protein/100 g. Cooked red meat contains 28–36 g/100 g, because the water content decreases and nutrients become more concentrated during cooking. The protein is highly digestible, around 94% compared with the digestibility of 78% in beans and 86% in whole wheat.[9] Protein from meat provides all essential amino acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine) and has no limiting amino acids. Protein Digestibility Corrected Amino Acid Score is a method of evaluating the protein quality, with a maximum possible score of 1.0. Animal meats like beef have a score of approximately 0.9, compared with values of 0.5–0.7 for most plant foods.[10] The amino acid glutamic acid/glutamine is present in meat in the highest amounts (16.5%), followed by arginine, alanine and aspartic acid.

Fat

Table 2 shows examples of the amount of separable fat found on typical retail cuts of red meat available for sale in Australia.[4, 11] There is a wide variation in the amount of total separable fat between the different beef and lamb cuts, ranging from 37% in loin lamb chops to only 1% in veal steak.

Table 2. Lean and separable fat from untrimmed raw boneless Australian red meat (mean weight)[4, 11]

Meat cut	*% Lean*	*% External fat*	*% Internal fat*
Beef			
Topside roast	91	6	3
Silverside roast	89	7	4
Blade steak	88	6	6
Porterhouse steak	77	18	5
Stir-fry	98	2	0
Scotch fillet	81	8	11
Veal			

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Leg steak	99	0	1
Diced	98	2	0
Cutlet	93	1	6
Lamb			
Leg roast	83	11	6
Chump chop	75	15	10
Diced	98	2	0

The gross composition values show that there generally appears to be less separable fat in the untrimmed raw retail samples collected in 2002 compared with those reported from 1983 to 1986.[12, 13] For example, the percentage separable fat has declined from 18% to 12% in rump steak and from 10% to 6.6% in fillet steak. This trend to lower-fat cuts has been due to three factors: selective breeding and feeding practices designed to increase the carcase lean-to-fat ratio; meat classification and marketing systems designed to favour leaner products; and modern butchery techniques such as seaming out whole muscles and trimming away intermuscular fat.[14]

Given the trend to prepare and consume meat after trimming external fat, the most recent nutritional analyses show that all trimmed lean red meats are relatively low in fat (<7%) and have moderate cholesterol content, with the exception of mince meats (Table 3). An important contributor to the leanness of muscle meat in Australian beef and lamb is that almost all animals are pasture (grass) fed for most of their lives, although some are given short periods of grain finishing before slaughter (D Thomason, MLA, personal communication).

Table 3. Fat and cholesterol content of trimmed raw and cooked Australian red meat (per 100 g)[5]

Meat cut	*Total fat (g)*	*Cholesterol (mg)*
Beef		
Diced, raw	2.7	54
Diced, cooked	3.0	77
Round steak, raw	1.7	62
Round steak, cooked	2.0	75
Topside roast, raw	4.7	35

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Topside roast, cooked	2.8	62
Sirloin steak, raw	1.9	58
Sirloin steak, cooked	3.8	70
Scotch fillet, raw	2.8	58
Scotch fillet, cooked	4.5	70
Regular mince, raw	10.8	76
Regular mince, cooked	12.7	99
Low-fat mince, raw	6.8	61
Low-fat mince, cooked	9.0	81

Fatty acids

While discussion on the fat content of red meat may focus on the saturated fat content, the amount of saturated fat in Australian beef and lamb is actually lower than the total amount of unsaturated fats on a per edible portion basis.

Table 4 shows the average fatty acid profiles of beef, veal, lamb and mutton compared with other white meats and fish. Saturated fatty acids comprise, on average, 40% of total fatty acids in the lean component and 48% in the fat component of red meat. In beef and veal, approximately half of the saturated fatty acid in both the lean and fat component of red meat is palmitic acid (16:0), and about a third is stearic acid (18:0). In lamb and mutton, the proportions of these two fatty acids are more similar. There is little variation between cuts in the proportion of fatty acids.

Table 4. Fatty acid profile of raw lean meats (g/100 g edible portion)

Fatty acid	*Beef* [(a)]	*Veal* [(a)]	*Lamb* [(a)]	*Mutton* [(a)]	*Skinless chicken* [(b)]	*Lean pork* [(c)]	*White fish* [(d)]	*Oily fish* [(e)]
C14:0	0.096	0.034	0.101	0.060	0.020	0.010	0.020	0.680
C15:0	0.012	0.006	0.016	0.011	0.000	0.000	0.000	0.070
C16:0	0.607	0.215	0.842	0.667	0.340	0.250	0.180	2.170
C17:0	0.028	0.009	0.051	0.036	0.010	0.000	0.000	0.050
C18:0	0.356	0.119	0.644	0.609	0.120	0.130	0.050	0.350

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Total saturated	1.149	0.409	1.730	1.464	0.500	0.400	0.300	3.320
C14:1	0.025	0.007	0.004	0.003	0.000	0.000	0.000	0.000
C16:1	0.082	0.033	0.066	0.039	0.004	0.030	0.060	0.590
C18:1	1.103	0.356	1.995	1.370	0.620	0.390	0.110	2.190
C20:1	0.015	0.048	0.010	0.011	0.010	0.010	0.010	1.340
Total monounsaturated	1.205	0.399	2.066	1.413	0.700	0.430	0.200	5.390
C18:2 ω-6	0.204	0.090	0.321	0.339	0.210	0.120	0.010	0.250
C18:3 ω-3	0.048	0.022	0.072	0.107	0.010	0.010	0.000	0.130
C20:3 ω-6	0.020	0.012	0.009	0.009	0.008	0.003	0.000	0.000
C20:4 ω-6	0.076	0.056	0.094	0.101	0.030	0.019	0.040	0.050

(a) Average values from 2002 analyses of Australian red meat.[15]

(b) Values for raw lean chicken breast from NUTTAB 2006.[16]

(c) Values for raw lean pork fillet from NUTTAB 2006.[16]

(d) Values for raw flathead from NUTTAB 2006.[16]

(e) Values for canned red salmon from NUTTAB 2006.[16]

DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid.

Polyunsaturated fatty acids (PUFA) range from 11% to 29% of total fatty acids. Pasture fed beef is a better source of omega-3 fats than grain feed beef, and this explains the better fatty acid ratio in Australian red meat compared with that in the USA, where there is extensive grain feeding.[17, 18] Beef and lamb also have more omega-3 fatty acids than either chicken or pork, although fish is still a significantly better source than any of the red meats.

The recent development of Nutrient Reference Values for Australians recommended a daily adequate intake of long-chain omega-3 fats (docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and docosapentaenoic acid) of 160 mg for men and 90 mg for women, with higher targets of 610 mg and 430 mg respectively to reduce the risk of long-term chronic disease.[8] As the levels of long-chain PUFA found in Australian beef, veal and lamb muscle meat are greater than 30 mg per serving (135 g) of red meat, they are considered a source of long-chain omega-3 PUFA according to Australian food regulations (Food Standards Australia New Zealand 2002)[1]. Mutton muscle meat, which has more than 60 mg EPA + DHA per serving of red meat, can be described as a good source of long-chain omega-3 polyunsaturated fats. Red meat is frequently consumed by Australians and makes the second greatest contribution to intake of long-chain omega-3 PUFA, after fish, in the Australian diet.[19]

Trans-fatty acids

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Trans-fatty acids are found in ruminant fat as a result of biohydrogenation by rumen bacteria. *Trans*-fatty acids (18:1 *trans*) in raw muscle meat vary from as little as 22 mg/100 g in veal to 123 mg/100 g in lamb, but is generally less than 3% of the total fatty acid content.[15] Levels in both raw and cooked muscle meat are higher in lamb and mutton than in beef and veal.

Choline

Choline is a precursor of a number of compounds, including neurotransmitters and membrane phospholipids. Although choline can be made in the body, dietary essentiality has been demonstrated, and the new Australian Nutrient Reference Values recommend an adequate intake of 550 mg/day for men and 425 mg/day for women.[8] The best dietary sources are milk, liver and eggs, but meat is also a significant source and beef contains 78 mg/100 g.[20]

Vitamins

As with other animal foods, red meat is an excellent source of bioavailable vitamin B12, providing over two-thirds of the daily requirement in a 100 g serve (Table 1). Up to 25% RDI of riboflavin, niacin, vitamin B6 and pantothenic acid can also be provided by 100 g of red meat, but compared with pork, it is a relatively poor source of thiamin. Liver is an excellent source of vitamin A and folate, but the levels in lean muscle meat tissue are low. For all these vitamins, older animals tend to have higher concentrations, so the levels in beef are generally higher than those in veal, and mutton has more than lamb. Levels of vitamin D in meat are low and difficult to measure and have often not been included in food composition data previously. However, recent assays of meat in New Zealand have reported levels of 0.10 μg vitamin D3 and 0.45 μg 25-OH D3 per 100 g in beef and levels of 0.04 and 0.93 μg/100 g respectively in lamb.[21] Given the higher biological activity of the 25-OH vitamin D, this means that 100 g of cooked beef could provide 12% of the estimated adequate intake of 10 μg/day for a 51- to 70-year-old individual,[8] and cooked lamb could provide more than 25%, and hence be an important source of this nutrient, especially for housebound elderly people.

Minerals

Beef and lamb meat are among the richest sources of the minerals iron and zinc, with 100 g providing at least one-quarter of daily adult requirements (Table 1). The iron in meat is mostly haem iron, which is well absorbed, and meat protein also appears to enhance the absorption of iron from meat. Similarly, absorption of zinc from a diet high in animal protein is greater than from plant foods, and the requirements for zinc may be as much as 50% higher for vegetarians.[8] Red meats are also good sources of selenium, providing over 20% RDI per 100 g serve, although it is likely that selenium values in meat will be significantly affected by where animals feed and the time of the year of sampling. Lean meat is low in sodium, with a potassium–sodium ratio of >5. The copper content in raw lean cuts range from 0.055 to

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0.190 mg/100 g in beef and veal, 0.090 to 0.140 mg/100 g in lamb, and 0.190 to 0.240 mg/100 g in mutton, all significantly higher than values reported in British meat.[22]

MEAT-BASED BIOACTIVE COMPOUNDS

In addition to the traditional essential nutrients with defined requirements, there are a number of meat-based bioactive substances that have been studied for their potential beneficial effects.[23]

Taurine

An amino acid in meat of particular interest is taurine. Meat is rich in taurine (110 mg/100 g in lamb and 77 mg/100 g in beef),[24] and is the most abundant dietary source. While taurine can be derived from methionine and cysteine metabolism, there have been suggestions that it should be considered a conditionally essential amino acid during lactation, during times of immune challenge, and may offer protection against oxidative stress.[25, 26]

Carnitine

L-carnitine (beta-hydroxy-gamma-trimethyl amino butyric acid) transports long-chain fatty acids across the inner mitochondrial membranes to produce energy during exercise. Although not an essential nutrient, needs appear to be increased during pregnancy and after strenuous exercise, and a recommended intake of 24–81 mg/day has been proposed.[27] It is found in skeletal muscle and is particularly abundant in sheep muscle at up to 209 mg/100 g[28] and in beef at around 60 mg/100 g.[29]

Conjugated linoleic acid

Conjugated linoleic acid (CLA) has antioxidant and immunomodulatory properties and may also play a role in the control of obesity.[30] As rumen bacteria convert linoleic acid to CLA, it is most abundant in the fat of ruminant animals, although CLA is also present in partially hydrogenated vegetable oils. The CLA content of meat is affected by several factors, including breed, age and food composition.[31] It is mostly present in the fat component of red meat (approximately 1 g/100 g) but is also found in the muscle meat: 10–46 mg/100 g in raw meat and 30–100 mg/100 g in cooked red meat.[15]

Endogenous antioxidants

Several endogenous compounds (including ubiquinone, glutathione, lipoic acid, spermine, carnosine, anserine) have been studied in skeletal muscle.[32] Both carnosine and anserine are antioxidative histidyl dipeptides and the most abundant antioxidants in meat. Carnosine is present at around 365 mg/100 g in beef[33] and 400 mg/100 g in lamb.[24] Because carnosine is absorbed into the plasma intact, it is a potentially important dietary antioxidant.[34]

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Coenzyme Q10 (ubiquinone) also has antioxidant properties, and supplements have shown beneficial effects in some studies.[35] Levels in meat are estimated to be around 2 mg/100 g in both beef and sheep meat.[33] Glutathione is a component of glutathione peroxidase enzymes, which have an important antioxidant role in the body. It may also play a role in immune response and enhancing iron absorption by contributing to the 'meat factor'. Glutathione levels in red meat are estimated to be 12–26 mg/100 g in beef,[36] and most meats contain approximately twice the level of glutathione of poultry and up to 10 times the content found in fish.

Creatine

Creatine and its phosphorylated derivative creatine phosphate play an important role in muscle energy metabolism and, under some circumstances, creatine supplements can enhance muscle performance.[37] Red meat contains approximately 350 mg/100 g[33] and is the principal dietary source for humans. Creatine in meat is readily absorbed,[38] but typical intakes are unlikely to provide the levels of creatine used for supplementation of sports performance (up to 15 g/day).

NUTRIENT COMPOSITION OF ORGAN MEATS

Table 5 provides a comparison of the nutrient content of liver, kidney, heart, brains and tripe from beef and lamb. From this table, the following general statements can be made:

Table 5. Selected nutrients (per 100 g) in raw liver, kidney, heart, brain and tripe [(a)]

	Liver		*Kidney*		*Heart*		*Brain*	*Tripe*
	Beef	*Lamb*	*Beef*	*Lamb*	*Beef*	*Lamb*	*Lamb*	*Beef*
Protein (g)	20.0	21.4	18.2	17.1	18.2	17.8	12.3	13.2
Fat (g)	8.6	7.5	1.6	2.5	3.0	5.6	8.0	2.1
Saturated fat (g)	2.8	2.2	0.6	0.9	1.2	2.3	2.2	0.9
Long-chain omega-3 fat (mg)	561	361	47	103	54	102	574	20
Cholesterol (mg)	271	433	313	338	103	129	1352	82
Thiamin (mg)	0.23	0.24	0.40	0.56	0.50	0.61	0.14	0
Riboflavin (mg)	4.80	2.80	3.60	2.10	1.50	1.10	0.40	0.10
Niacin (mg)	9.4	10.9	6.5	7.6	6.9	5.9	5.1	0.2
Folate (μg)	290	230	98	28	3	2	3	5

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Vitamin B12 (μg)	59	90	28	52	9	10	11	1
Retinol equivalents (μg)	13 877	31 400	155	93	10	0	0	0
Zinc (mg)	3.6	4.3	1.8	2.6	1.6	1.6	1.1	1.2
Iron (mg)	5.8	9.5	5.4	9.8	5.0	3.9	1.7	0.4
Magnesium (mg)	15	19	15	16	17	17	12	6

(a) Folate values from US data;[39] all other values from NUTTAB 2006.[16]

- All organ meats (except tripe) are extremely rich in vitamin B12, with much more than 100% of the RDI in 100 g
- Liver is a rich source of protein, iron, zinc, riboflavin, niacin, vitamin A and folate
- Kidney is rich in protein, thiamin, riboflavin, iron and a source of folate
- Heart is a good source of iron and zinc, but not as good as liver and kidney
- Brains and tripe are not particularly good sources of vitamins or minerals
- All organ meats are high in cholesterol, especially brains, and mostly low in sodium
- Liver is such a rich source of retinol that consumption of large amounts is not recommended in pregnancy.[40]

SUMMARY

The Australian Guide to Healthy Eating recommends that a healthy diet include 1–2 serves per day of meat or equivalents such as eggs, nuts or legumes.[41] However, the vegetarian alternatives that are used as protein sources have very different nutritional profiles from red meat, as shown in Table 6, which compares the percentage of an adult male daily requirement provided by 100 g of food. Lean beef and lamb are better protein sources than all the options except cheese, and are mostly lower in sodium. The meats are higher in zinc and niacin than all the alternatives, and also higher in omega-3 fats than the vegetable sources; a better source of vitamin B6 (except for the walnuts); richer in selenium (except in comparison to eggs); and are the best source of vitamin B12, which is absent entirely from the vegetable products. Egg, cheese and nuts are also much higher sources of total fat than lean beef or lamb. Thus, data on the nutritional composition of lean red meat highlight the relative value of this food category in delivering essential nutrients in a reasonably balanced form.

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Table 6. Percentage of male adult recommended dietary intake (RDI) or adequate intake (AI) provided by 100 g of lean red meat and some vegetarian protein sources

	RDI/AI for men aged 31–50 years	*Beef* [(a)] *% of RDI*	*Lamb* [(a)] *%*	*Egg* [(b)] *%*	*Cheddar cheese* [(b)]	*Baked beans salt reduced* [(b)]	*Walnuts* [(b)] *%*
Protein	64 g	36	34	21	40	7	23
Long-chain omega-3 fat	160 mg	50	53	111	55	0	0
Thiamin	1.2 mg	3	8	8	3	4	28
Riboflavin	1.3 mg	25	15	31	39	0	14
Niacin	16 mg	31	70	0	<1	5	9
Vitamin B6	1.3 mg	23	43	5	6	8	33
Vitamin B12	2.4 μg	79	71	58	35	0	0
Pantothenic acid	6 mg	12	13	34	7	<1	11
Vitamin A	900 μg	<1	<1	25	43	<1	<1
Vitamin E	10 mg	7	5	22	40	1	26
Phosphorus	1000 mg	22	23	20	47	8	37
Zinc	14 mg	30	31	9	26	4	18
Iron	8 mg	24	25	26	3	20	31

(a) Average values from 2002 analyses of Australian red meat.[5] (b) Values from NUTTAB 2006.[16]

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336    ##$atext $btxt $2rdacontent
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362    1#$aBegan with: Vol. 59, no. 1 (Mar. 2002).
500    ##$aRefereed/Peer-reviewed
500    ##$aHas supplements.
550    ##$aJournal of: Dietitians Association of Australia,
including Dietitians New Zealand, <2010->
588    0#$aVol. 62, issue 1 (Mar. 2005); title from table of
contents page (Wiley-Interscience, viewed June 26, 2009).
588    1#$aVol. 78, issue 1 (February 2021) (Wiley Online Library,
viewed February 19, 2021).
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906    ##$aJOURNAL
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Attachment 52c

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

LC control no.: sh 86005508
LCCN Permalink: https://lccn.loc.gov/sh86005508
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670 __ |**a** Work cat.: Helm, K.K. The entrepreneurial nutritionist, c1987.
670 __ |**a** Swanson, B. Careers in health care, 1984: pp. 63-69.
670 __ |**a** Dorland's med. dict. |**b** ("Science or study and regulation of the diet")
670 __ |**a** Gould's med. dict.
670 __ |**a** Stedman's med. dict. |**b** (Branch of therapeutics treating of food and drink in relation to health and diseases)
670 __ |**a** Web. 3 |**b** (Science or art of applying principles of nutrition to the feeding of individuals or groups under different economic conditions or for hygienic or therapeutic purposes)
670 __ |**a** MESH.
670 __ |**a** NIH |**b** (Nutrition, Dietetics)
670 __ |**a** TEST.
906 __ |**t** 8639 |**u** fk02 |**v** 1
952 __ |**a** 84 bib. records to be changed
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RESEARCH ARTICLE

A Prospective Study of Red and Processed Meat Intake in Relation to Cancer Risk

Amanda J Cross, Michael F Leitzmann, Mitchell H Gail, Albert R Hollenbeck, Arthur Schatzkin, Rashmi Sinha

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Abstract

Background

Red meat and processed meat have been associated with carcinogenesis at several anatomic sites, but no prospective study has examined meat intake in relation to a range of malignancies. We investigated whether red or processed meat intake increases cancer risk at a variety of sites.

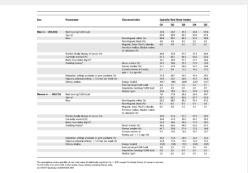
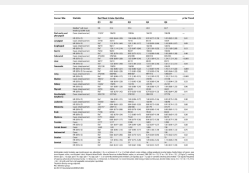
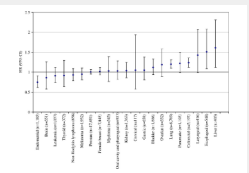
Methods and Findings

The National Institutes of Health (NIH)-AARP (formerly the American Association for Retired Persons) Diet and Health Study is a cohort of approximately 500,000 people aged 50–71 y at baseline (1995–1996). Meat intake was estimated from a food frequency questionnaire administered at baseline. Cox proportional hazards regression was used to estimate hazard ratios and 95% confidence intervals within quintiles of red and processed meat intake. During up to 8.2 y of follow-up, 53,396 incident cancers were ascertained. Statistically significant elevated risks (ranging from 20% to 60%) were evident for esophageal, colorectal, liver, and lung cancer, comparing individuals in the highest with those in the lowest quintile of red meat intake. Furthermore, individuals in the highest quintile of processed meat intake had a 20% elevated risk for colorectal and a 16% elevated risk for lung cancer.

Conclusions

Both red and processed meat intakes were positively associated with cancers of the colorectum and lung; furthermore, red meat intake was associated with an elevated risk for cancers of the esophagus and liver.

Figures

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Competing interests: The authors have declared that no competing interests exist.

Abbreviations: AARP, formerly known as the American Association for Retired Persons; BMI, body mass index; CI, confidence interval; FFQ, food frequency questionnaire; HR, hazard ratio; HCA, heterocyclic amine; NAT, N-acetyltransferase; NOC, N-nitroso compound; PAH, polycyclic aromatic hydrocarbon; RR, relative risk

Subject Areas

- Meat
- Lung and intrathoracic t...
- Colorectal cancer
- Cancer risk factors
- Prostate cancer
- Breast cancer
- Esophageal cancer
- Diet

Editors' Summary

Background.

Every year, there are more than 10 million new cases of cancer around the world. These cases are not spread evenly across the globe. The annual incidence of cancer (the number of new cases divided by the population size) and the type of cancer most commonly diagnosed varies widely among countries. Much of the global variation in cancer incidence and type is thought to be due to environmental influences. These include exposure to agents in the air or water that cause cancer, and lifestyle factors such as smoking and diet. Researchers identify environmental factors that affect cancer risk by measuring the exposure of a large number of individuals to a specific environmental factor and then monitoring these people for several years to see who develops cancer. The hope is that by identifying the environmental factors that cause or prevent cancer, the global burden of cancer can be reduced.

Why Was This Study Done?

Diet is thought to influence the incidence of several cancers but it is very difficult to unravel which aspects of diet are important. Being overweight, for example, is strongly associated with an increased risk of developing several types of cancer, but the evidence that the intake of red meat (beef, pork, and lamb) and of processed meat (for example, bacon, ham, and sausages) is linked to cancer risk is much weaker. Although several studies have linked a high intake of red meat and processed meat to an increased risk of colorectal cancer (the colon is the large bowel; the rectum is the final few inches of the large bowel before the anus), whether this aspect of diet affects the

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risk of other types of cancer is unclear. In this prospective study, the researchers have examined the association between meat intake and the incidence of a wide range of cancers.

What Did the Researchers Do and Find?

In 1995–1996, nearly half a million US men and women aged 50–71 y joined the NIH-AARP Diet and Health Study. The participants in this study—none of whom had had cancer previously—completed a questionnaire about their dietary habits over the previous year and provided other personal information such as their age, weight, and smoking history. The researchers used these data and information from state cancer registries to look for associations between the intake of red and processed meat and the incidence of various cancers. They found that people whose red meat intake was in the top fifth of the range of intakes recorded in the study (the highest quintile of consumption) had an increased risk of developing colorectal, liver, lung, and esophageal cancer when compared with people in the lowest quintile of consumption. People in the highest quintile of processed meat intake had an increased risk of developing colorectal and lung cancer. The incidences of other cancers were largely unaffected by meat intake.

What Do These Findings Mean?

These findings provide strong evidence that people who eat a lot of red and processed meats have greater risk of developing colorectal and lung cancer than do people who eat small quantities. They also indicate that a high red meat intake is associated with an increased risk of esophageal and liver cancer, and that one in ten colorectal and one in ten lung cancers could be avoided if people reduced their red and processed meat intake to the lowest quintile. However, although the researchers allowed for factors such as smoking history that might have affected cancer incidences, some of the effects they ascribe to meat intake might be caused by other lifestyle factors. Furthermore, because the study's definitions of red meat and processed meat overlapped—bacon and ham, for example, were included in both categories—exactly which type of meat is related to cancer remains unclear. Finally, most of the study participants were non-Hispanic white, so these findings may not apply to people with different genetic backgrounds. Nevertheless, they add to the evidence that suggests that decreased consumption of red and processed meats could reduce the incidence of several types of cancer.

Additional Information.

Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0040325.

- The American Cancer Society provides answers to common questions about diet and cancer
- Information is available from the charity Cancer Research UK about diet, healthy eating, and cancer
- The American Institute for Cancer Research also provides information on diet and cancer
- The NIH-AARP Diet and Health Study presents information on the impact of diet and lifestyle factors on risk of cancer
- The US National Cancer Institute provides information about the kind of food questionnaire used in this study

Introduction

Much of the global variation in cancer incidence has been attributed to environmental influences, including dietary preferences. Not only does meat intake vary substantially around the world, but diets high in red or processed meats have been associated with carcinogenesis at a variety of anatomic sites. The evidence to support a positive association between meat intake and carcinogenesis is based on an assortment of research ranging from laboratory studies [1–3] to observational epidemiology [4–8].

Thus far, the majority of epidemiologic meat research has focused on the more prevalent cancers, particularly colorectal cancer. The most recent meta-analysis of prospective studies of meat and colorectal cancer reported significantly elevated summary relative risks (RRs) for both red meat (RR = 1.28; 95% confidence interval (CI) = 1.15–1.42) and processed meat (RR = 1.20; 95% CI = 1.11–1.31) in the highest versus lowest category of intake [9]. To date, findings for other major cancers—such as prostate, breast, lung, and pancreatic cancer—are less consistent. Prospective data for rarer cancers are sparse, and most epidemiologic studies of

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less–commonly occurring cancers are restricted to case-control studies, for which investigations of dietary associations are difficult, because of the potential for recall bias [10].

We prospectively investigated red and processed meat intake in relation to cancer incidence in a cohort of approximately half a million men and women enrolled in the National Institutes of Health (NIH)-AARP (formerly known as the American Association for Retired Persons) Diet and Health Study. This study's large size facilitated the investigation of comparatively rare cancers that have not yet been prospectively investigated.

Materials and Methods

Study Population

The NIH-AARP Diet and Health Study is a large prospective cohort of men and women, aged 50–71 y, from six states in the United States (California, Florida, Louisiana, New Jersey, North Carolina, and Pennsylvania) and two metropolitan areas (Atlanta, Georgia; and Detroit, Michigan). Recruitment began in 1995 when a self-administered questionnaire eliciting information on demographic and lifestyle characteristics, including dietary habits, was mailed to 3.5 million members of AARP; further details of the design of the study have been described in detail elsewhere [11,12]. The NIH-AARP Diet and Health Study was approved by the Special Studies Institutional Review Board of the US National Cancer Institute, and written informed consent was obtained from all participants by virtue of completing the baseline questionnaire.

Dietary Assessment

A 124-item food frequency questionnaire (FFQ), based on the Diet History Questionnaire (http://riskfactor.cancer.gov/DHQ/forms/files/shared/dhq1.2002.sample.pdf), developed at the National Cancer Institute, was completed at baseline. The FFQ assessed the usual frequency of consumption and portion size information (based on three categories of <25th, 25th–75th, and >75th percentile of intake from national dietary data) of foods and drinks over the previous 12 mo. Portion sizes and daily nutrient intakes were calculated from the 1994–1996 US Department of Agriculture's Continuing Survey of Food Intake by Individuals [13]. The FFQ compared favorably to other FFQs [14], and was also calibrated within this study population against two 24-h recall diaries [12]. Red meat intake was calculated from the frequency of consumption and portion size information of all types of beef, pork, and lamb; this included bacon, beef, cold cuts, ham, hamburger, hot dogs, liver, pork, sausage, and steak. The processed meat variable included bacon, red meat sausage, poultry sausage, luncheon meats (red and white meat), cold cuts (red and white meat), ham, regular hot dogs, and low-fat hot dogs made from poultry. The meat variables also included meats added to complex food mixtures, such as pizza, chili, lasagna, and stew.

Cohort Follow-Up and Case Ascertainment

Cohort members are followed annually for change of address using the US Postal Service, and vital status is ascertained by annual linkage to the US Social Security Administration Death Master File. Follow-up for these analyses was calculated from baseline (1995–1996) until censoring at the end of 2003, or when the participant moved out of one of the eight study areas, had a cancer diagnosis, or died, whichever came first. Cancer cases were identified by linkage to state cancer registries and the National Death Index Plus. The eight state cancer registry databases are estimated to be 95% complete within 2 y of cancer incidence and are certified by the North American Association of Central Cancer Registries for meeting the highest standard of data quality [11]. Cancer diagnoses contributed to the incidence of the tumor site of the first diagnosis only and not subsequent diagnoses for additional sites. The cancer endpoints were defined by anatomic site and histologic code of the International Classification of Diseases for Oncology (ICD-0–3) [15]. Advanced prostate cancer may have a distinct etiology; therefore, we used the Tumor-Node-Metastasis classification system [16], and defined those with clinical or pathologic stage T3, T4, or N1 or M1, as well as individuals who died of prostate cancer, to have advanced disease. Results are presented for cancer sites with at least 60 cases within each sex.

Statistical Analysis

A total of 567,169 persons returned the baseline questionnaire and were available for analysis. We excluded individuals with duplicate records (n = 179), those who died before returning the baseline questionnaire (n = 261), those who had zero person years of follow-up (n = 32), those who moved out of the eight study areas before returning the questionnaire (n = 269), those who requested to be withdrawn from the study (n = 1), those whose questionnaire was filled in by someone else on their behalf (n = 15,765), those who had prevalent cancer (as noted by cancer registry or self-report) at baseline (n = 51,212) or end-stage renal disease (n = 997), and those with extreme daily total energy intake (n = 4,417), defined as more than two inter-quartile ranges above the 75th or below the 25th percentile on the logarithmic scale. After all

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exclusions, our analytic cohort consisted of 494,036 persons (294,724 men and 199,312 women).

Hazard ratios (HRs) and 95% CIs were estimated using Cox proportional hazards regression with time since entry (person years) as the underlying time metric. Analyses using age as the underlying time metric yielded almost identical HRs. We controlled for age as a continuous variable in the Cox model; inclusion of a quadratic term for age did not improve the fit of the model, nor did it modify the HRs appreciably. Multivariate HRs are reported within quintiles, using the lowest quintile as the referent category, adjusted for the covariates listed in the tables. Missing data were minimal for this study; where appropriate—i.e., for smoking history and body mass index (BMI)—we created "missing" categories, but for others, such as family history of cancer, we set individuals missing this data to zero: no family history. Saturated fat intake and menopausal hormone therapy were investigated as potential confounders of the meat–cancer association, but neither proved to change the HR by greater than 10% and were not, therefore, included in the final models. Extremely fine control for smoking history and dosage was investigated, by constructing a 31-level variable using smoking status (never, former, current), time since quitting for former smokers, and smoking dose.

We examined models that were adjusted for energy by two different methods: the multivariate nutrient density method and the residual energy adjustment method [10]. Since both methods of energy adjustment gave similar results in our study, and because the interpretation of the multivariate nutrient density method applies to actual intakes as a percent of energy, which tends to be the units expressed for public health recommendations, we report the results from the multivariate nutrient density adjusted models.

Risks are reported for both sexes combined, unless there was a statistically significant interaction between meat intake and sex. Interactions were evaluated by including cross product terms in multivariate models. Furthermore, we conducted a lag analysis, excluding the first 2 y of follow-up, as well as various subanalyses to verify the stability of our risk estimates within subgroups of race, BMI, education, smoking status, physical activity level, and alcohol intake. The proportional hazards assumption was verified using a time interaction model. Tests for linear trend were calculated using the median value of each quintile. All reported *p*-values are two-sided. To test for heterogeneity, we used a chi-square test. To test for heterogeneity among the 21 cancer sites with 20 degrees of freedom, we first calculated the weighted average of the 21 beta coefficients from the Cox model, with weights being proportional to the inverse of the variances. Then we calculated the following chi-square statistic: $T = \sum_{i=1}^{21} (\hat{\beta}_i - \bar{\beta})^2 / \sigma_i^2$, where $\hat{\beta}_i$ and σ_i^2 are the coefficient and its variance for each cancer, and $\bar{\beta}$ is the weighted average of the beta coefficients. Likewise, to test for heterogeneity between subsites, such as colon versus rectum, we used the same chi-squared test given above, but using one degree of freedom. We also calculated population-attributable risks as an estimate of the percentage of cases that could be prevented if individuals adopted intake levels within the first quintile, given the assumption of a causal association between red or processed meat intake and cancer. All statistical analyses were carried out using Statistical Analytic Systems (SAS) software (SAS Institute).

Results

During a mean follow-up of 6.8 y, 53,396 cancer diagnoses (36,907 male cases and 16,489 female cases) were ascertained. The mean energy–adjusted red meat intake in this cohort was 34.6 g/1,000 kcal (38.0 g/1,000 kcal in men and 29.5 g/1,000 kcal in women). The medians of extreme quintiles ranged from 9.8 to 62.7 g/1,000 kcal for red meat and 1.6 to 22.6 g/1,000 kcal for processed meat.

In general, those in the highest quintile of red meat intake tended to be slightly younger, less educated, less physically active, and less likely to consume fruits, vegetables, and alcohol than those in the lowest quintile. In contrast, those in the highest quintile of red meat intake were more likely to have a higher total energy intake, a higher BMI, and more likely to be a current smoker. Women in the highest quintile of red meat intake were also more likely to be married than those in the lowest quintile (Table 1).

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Motif Exhibit 1011, Page 907 of 1241

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Sex	Parameter	Characteristics	Quintile Red Meat Intake				
			Q1	Q2	Q3	Q4	Q5
Men (*n* = 284,724)	Red meat (g/1,000 kcal)		13.9	24.7	35.1	45.8	67.8
	Age (y)		63.6	62.5	62.2	62.0	61.4
	Race	Non-Hispanic white (%)	89.0	92.1	93.2	94.0	93.9
		Non-Hispanic black (%)	4.0	3.3	2.6	2.0	1.9
		Hispanic, Asian, Pacific Islander, American Indian, Alaskan native, or unknown (%)	6.9	4.9	4.2	3.7	4.2
	Positive family history of cancer (%)		46.0	47.0	47.1	47.3	46.8
	Currently married (%)		81.3	85.1	86.4	86.6	85.4
	Body mass index (kg/m^2)		26.1	26.9	27.3	27.7	28.4
	Smoking history[a]	Never smoker (%)	34.5	30.6	29.3	27.8	25.8
		Former smoker (%)	57.1	57.8	56.0	56.1	54.8
		Current smoker or having quit < 1 y ago (%)	6.7	9.9	12.3	14.2	17.5
	Education, college graduate or post graduate (%)		51.8	46.7	43.9	41.6	38.5
	Vigorous physical activity, ≥ 5 times per week (%)		29.4	22.7	20.0	18.1	16.8
	Dietary intake	Energy (kcal/d)	1911	1963	2009	2058	2121
		Fruit (servings/1,000 kcal)	2.2	1.7	1.5	1.2	1.1
		Vegetables (servings/1,000 kcal)	2.3	2.0	2.0	2.0	1.9
		Alcohol (g/d)	20.6	19.3	18.7	18.8	13.2
Women (*n* = 199,312)	Red meat (g/1,000 kcal)		7.8	17.6	26.3	36.3	54.7
	Age (y)		62.1	62.1	62.0	61.7	61.3
	Race	Non-Hispanic white (%)	85.2	88.7	90.2	91.4	91.3
		Non-Hispanic black (%)	8.1	6.2	5.4	4.3	4.0
		Hispanic, Asian, Pacific Islander, American Indian, Alaskan native, or unknown (%)	6.7	5.3	4.4	4.3	4.7
	Positive family history of cancer (%)		50.0	51.6	51.7	51.9	50.5
	Currently married (%)		56.6	61.8	65.0	65.3	63.5
	Body mass index (kg/m^2)		25.8	26.4	26.9	27.6	28.2
	Smoking history[a]	Never smoker (%)	46.6	45.6	44.0	43.6	41.5
		Former smoker (%)	41.7	38.0	37.4	35.8	34.8
		Current smoker or having quit < 1 y ago (%)	9.7	13.5	16.0	18.7	22.7
	Education, college graduate or post graduate (%)		30.2	31.8	29.0	26.6	23.3
	Vigorous physical activity, ≥ 5 times per week (%)		22.9	17.6	15.0	12.9	11.2
	Dietary intake	Energy (kcal/d)	1528	1530	1517	1508	1539
		Fruit (servings/1,000 kcal)	3.6	2.2	1.9	1.6	1.4
		Vegetables (servings/1,000 kcal)	2.9	2.5	2.4	2.3	2.2
		Alcohol (g/d)	5.5	6.2	6.3	5.9	5.2

The associations across quintiles of red meat were all statistically significant ($p < 0.01$), except for family history of cancer in women.
[a]3,173 (1.9%) men and 3,794 (2.0%) women have missing smoking history data.
doi:10.1371/journal.pmed.0040325.t001

Table 1.
Means and Proportions for Baseline Characteristics of the NIH-AARP Diet and Health Study Cohort (*n* = 494,036) by Red Meat Quintile
https://doi.org/10.1371/journal.pmed.0040325.t001

Red Meat

Individuals in the highest quintile of red meat intake, compared with those in lowest, had a statistically significant elevated risk of several malignancies (Table 2), including esophageal (HR = 1.51; 95% CI = 1.09–2.08; *p* for trend = 0.13), colorectal (HR = 1.24; 95% CI = 1.12–1.36; *p* for trend < 0.001, liver (HR = 1.61; 95% CI = 1.12–2.31; *p* for trend = 0.04), lung (HR = 1.20; 95% CI = 1.10–1.31; *p* for trend < 0.001), and borderline statistical significance for laryngeal cancer (HR = 1.43; 95% CI = 0.99–2.07; *p* for trend = 0.09). The positive association for red meat intake and colorectal cancer was due more to cancer of the rectum (*n* = 1,418; HR = 1.45; 95% CI = 1.20–1.75; *p* for trend < 0.001) than the colon (*n* = 3,689; HR = 1.17; 95% CI = 1.05–1.31; *p* for trend < 0.001), and this difference in risk was marginally statistically significant (*p* for heterogeneity = 0.06). Additional fine control for smoking did not alter the associations for cancers of the esophagus, colorectum, liver, lung, or larynx. In addition, the tests for interaction between smoking and both red meat (*p* interaction = 0.69) and processed meat (*p* interaction = 0.48) intake for lung cancer risk were not statistically significant. The population-attributable risks, representing the percentage of cases that could be prevented if individuals adopted red meat intake levels within the first quintile, were 33%, 9%, 35%, and 10% for esophageal, colorectal, liver, and lung cancer, respectively.

Cancer Site	Statistic	Red Meat Intake Quintiles					p for Trend
		Q1	Q2	Q3	Q4	Q5	
	Median[a] red meat intake (g/1,000 kcal)	5.8	21.4	31.4	42.9	62.7	—
Oral cavity and pharyngeal	Cases (men/women)	11/137	136/93	139/56	144/93	196/38	—
	HR (95% CI)	Ref	0.80 (0.64–1.00)	1.04 (0.84–1.29)	0.95 (0.77–1.19)	1.03 (0.82–1.28)	0.41
Laryngeal	Cases (men/women)	37/10	45/13	73/16	85/18	103/9	—
	HR (95% CI)	Ref	1.12 (0.76–1.64)	1.33 (1.00–2.17)	1.43 (0.99–2.06)	1.43 (0.99–2.07)	0.09
Esophageal	Cases (men/women)	48/14	96/17	92/11	103/18	120/12	—
	HR (95% CI)	Ref	1.56 (1.14–2.14)	1.33 (0.97–1.84)	1.30 (1.00–1.92)	1.51 (1.09–2.08)	0.13
Gastric	Cases (men/women)	72/42	90/51	90/37	130/32	158/32	—
	HR (95% CI)	Ref	1.02 (0.76–1.32)	0.92 (0.75–1.21)	1.14 (0.88–1.48)	1.08 (0.83–1.38)	0.48
Colorectal	Cases (men/women)	472/418	578/546	674/299	830/310	905/255	—
	HR (95% CI)	Ref	0.96 (0.89–1.08)	1.03 (0.94–1.14)	1.20 (1.09–1.31)	1.24 (1.13–1.36)	<0.001
Liver	Cases (men/women)	30/17	56/13	65/19	68/20	89/13	—
	HR (95% CI)	Ref	1.33 (0.93–1.90)	1.49 (1.04–2.13)	1.53 (1.05–2.16)	1.61 (1.12–2.31)	0.04
Pancreatic	Cases (men/women)	105/108	118/93	126/88	149/77	226/70	—
	HR (95% CI) for men	Ref	1.02 (0.80–1.31)	1.02 (0.80–1.22)	1.12 (0.87–1.44)	1.43 (1.15–1.83)	0.001
	HR (95% CI) for women	Ref	0.92 (0.66–1.28)	1.13 (0.81–1.56)	1.25 (0.90–1.74)	0.92 (0.64–1.32)	0.92
Lung	Cases (men/women)	474/306	608/366	890/467	957/512	1380/437	—
	HR (95% CI)	Ref	1.07 (0.96–1.17)	1.11 (1.02–1.21)	1.16 (1.07–1.27)	1.20 (1.10–1.31)	<0.001
Bladder	Cases (men/women)	180/37	251/38	282/53	290/45	372/38	—
	HR (95% CI)	Ref	1.08 (0.91–1.28)	1.13 (0.92–1.28)	1.05 (0.88–1.24)	1.12 (0.94–1.33)	0.33
Kidney	Cases (men/women)	134/87	178/70	192/78	225/70	267/67	—
	HR (95% CI)	Ref	1.07 (0.89–1.28)	1.05 (0.88–1.24)	1.06 (0.88–1.27)	1.04 (0.86–1.25)	0.96
Thyroid	Cases (men/women)	33/53	33/56	33/29	24/38	48/21	—
	HR (95% CI)	Ref	1.05 (0.70–1.42)	0.79 (0.56–1.18)	0.79 (0.56–1.13)	0.90 (0.64–1.30)	0.41
Non-Hodgkin lymphoma	Cases (men/women)	202/159	227/160	279/132	280/123	277/76	—
	HR (95% CI)	Ref	1.04 (0.90–1.21)	1.09 (0.94–1.27)	1.08 (0.93–1.26)	0.93 (0.79–1.09)	0.30
Leukemia	Cases (men/women)	123/80	138/51	144/53	166/44	186/48	—
	HR (95% CI)	Ref	0.84 (0.60–1.03)	0.84 (0.60–1.03)	0.86 (0.73–1.06)	0.91 (0.74–1.12)	0.68
Melanoma	Cases (men/women)	234/157	239/110	280/98	341/79	315/77	—
	HR (95% CI)	Ref	0.84 (0.73–0.98)	0.90 (0.78–1.04)	0.90 (0.80–1.14)	0.95 (0.83–1.11)	0.54
Brain	Cases (men/women)	71/36	67/46	94/33	78/23	80/23	—
	HR (95% CI)	Ref	1.02 (0.70–1.34)	1.12 (0.85–1.47)	0.89 (0.67–1.19)	0.83 (0.63–1.15)	0.26
Myeloma	Cases (men/women)	61/51	60/40	75/41	85/35	50/19	—
	HR (95% CI)	Ref	0.88 (0.67–1.17)	1.04 (0.79–1.36)	0.96 (0.72–1.28)	1.01 (0.73–1.39)	0.59
Prostate	Cases	3,777	3,157	3,508	3,803	3,998	—
	HR (95% CI)	Ref	1.01 (0.97–1.06)	1.04 (0.99–1.09)	1.02 (0.97–1.07)	1.01 (0.96–1.07)	0.58
Female breast	Cases	1,465	1,251	1,202	1,806	738	—
	HR (95% CI)	Ref	1.05 (0.96–1.14)	1.07 (0.95–1.17)	1.05 (1.00–1.19)	1.02 (0.93–1.12)	0.75
Endometrial	Cases	333	274	286	204	198	—
	HR (95% CI)	Ref	0.83 (0.67–0.96)	0.83 (0.75–1.01)	0.78 (0.64–0.94)	0.75 (0.63–0.91)	0.02
Ovarian	Cases	149	131	92	110	73	—
	HR (95% CI)	Ref	1.21 (0.92–1.50)	0.97 (0.73–1.28)	1.01 (0.82–1.45)	1.13 (0.80–1.59)	0.33
Cervical	Cases	29	25	27	16	28	—
	HR (95% CI)	Ref	0.64 (0.33–1.23)	1.09 (0.61–1.96)	1.02 (0.55–1.87)	1.06 (0.57–1.94)	0.44

Multivariate model includes: age (continuous), sex, education (<8 y or unknown, 8–11 y, 12 y (high school), some college, college graduate), marital status, family history of cancer, race (non-Hispanic white, non-Hispanic black, Hispanic/Asian/Pacific Islander/American Indian/Alaskan native, or unknown), BMI (18.5 to <25, 25 to <30, 30 to <35, ≥35 kg/m^2), smoking (never, quit ≥ 10y ago, quit 5–9 y ago, quit 1–4 y ago, quit < 1y or currently smoking and smoked ≥20 cig/day, quit < 1y ago or currently smoking and smoked <20 cig/day), frequency of vigorous physical activity (never/rarely, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5 or more times/wk), total energy intake (continuous), alcohol intake (none, 0 to <5, 5 to <15, 15 to <30, ≥30 g/d), and fruit and vegetable consumption (quintiles).
[a]Nutrient density energy adjusted.
Ref, reference group.
doi:10.1371/journal.pmed.0040325.t002

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Table 2.
Multivariate HRs and 95% CIs (Both Sexes Combined) for Red Meat Intake and Cancer in the NIH-AARP Diet and Health Study
https://doi.org/10.1371/journal.pmed.0040325.t002

Red meat intake was not associated with gastric or bladder cancer, leukemia, lymphoma, or melanoma. The associations between red meat and cancer are summarized in Figure 1, arranged by order of the magnitude of the risk; the figure also shows the null findings for sex-

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specific cancers, such as breast, ovarian, cervical, and prostate cancer. Unexpectedly, red meat intake was inversely associated with endometrial cancer (HR = 0.75; 95% CI = 0.62–0.91; *p* for trend = 0.02).

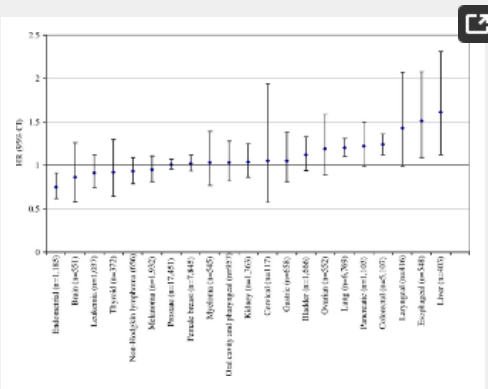

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Figure 1. HRs and 95% CIs for the 5th Versus 1st Quintile of Red Meat Intake and Cancer Risk for Both Sexes Combined (Except for Sex-Specific Cancers)
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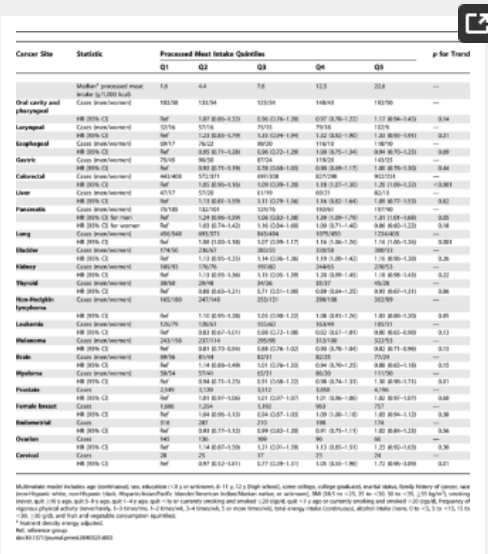
In further sex-specific analyses, red meat intake was positively associated with pancreatic cancer among men only (HR = 1.43; 95% CI = 1.11–1.83; *p* for trend = 0.001; *p* interaction by sex = 0.03); this risk was not attenuated by fine control for smoking. We observed no differences in risk by sex for other cancer sites.

Processed Meat

Individuals in the highest quintile, compared with those in the lowest quintile, of processed meat intake were at elevated risk for colorectal (HR = 1.20; 95% CI = 1.09–1.32; *p* for trend < 0.001) and lung cancer (HR = 1.16; 95% CI = 1.06–1.26; *p* for trend = 0.001) (Table 3). In concordance with the red meat association, the risk observed for processed meat and colorectal cancer was slightly higher for rectal (HR = 1.24; 95% CI = 1.03–1.49; *p* for trend = 0.03) than colon cancer (HR = 1.18; 95% CI = 1.06–1.32; *p* for trend = 0.001), although this difference in risk was not statistically significant (*p* for heterogeneity = 0.67). Additional fine control for smoking did not alter the risk estimates for cancers of the colorectum or lung. Furthermore, borderline statistically significant increased risks for bladder cancer (HR = 1.16; 95% CI = 0.98–1.38; *p* for trend = 0.26) and myeloma (HR = 1.30; 95% CI = 0.98–1.71; *p* for trend = 0.01) were observed for those in the highest quintile of processed meat intake. The population-attributable risks, representing the proportion of cases potentially preventable if individuals adopted processed meat intake levels within the first quintile, were 10% for colorectal cancer and 9% for lung cancer.



Cancer Site	Statistic	Processed Meat Intake Quintiles					p for Trend
		Q1	Q2	Q3	Q4	Q5	
	Median[a] processed meat intake (g/1,000 kcal)	1.6	4.4	7.6	12.3	22.6	—
Oral cavity and pharyngeal	Cases (men/women)	183/58	133/54	123/54	148/43	183/50	—
	HR (95% CI)	Ref	1.07 (0.85–1.32)	0.96 (0.76–1.20)	0.97 (0.76–1.22)	1.17 (0.94–1.45)	0.14
Laryngeal	Cases (men/women)	57/16	57/16	75/15	79/16	103/6	—
	HR (95% CI)	Ref	1.23 (0.83–1.79)	1.33 (0.94–1.94)	1.22 (0.82–1.80)	1.43 (0.90–1.91)	0.21
Esophageal	Cases (men/women)	69/17	76/22	90/20	116/12	118/10	—
	HR (95% CI)	Ref	0.95 (0.71–1.28)	0.96 (0.72–1.28)	1.09 (0.75–1.34)	0.94 (0.70–1.23)	0.69
Gastric	Cases (men/women)	75/45	86/50	87/34	118/28	143/33	—
	HR (95% CI)	Ref	0.92 (0.71–1.19)	0.78 (0.58–1.05)	0.98 (0.69–1.17)	1.00 (0.78–1.30)	0.44
Colorectal	Cases (men/women)	443/408	572/371	690/308	827/298	902/314	—
	HR (95% CI)	Ref	1.05 (0.90–1.16)	1.09 (0.09–1.28)	1.18 (1.07–1.30)	1.20 (1.09–1.32)	<0.001
Liver	Cases (men/women)	47/17	57/20	61/19	69/21	83/10	—
	HR (95% CI)	Ref	1.13 (0.81–1.59)	1.11 (0.79–1.56)	1.16 (0.82–1.64)	1.00 (0.77–1.33)	0.82
Pancreatic	Cases (men/women)	79/180	182/101	126/78	192/61	157/90	—
	HR (95% CI) for men	Ref	1.24 (0.96–1.59)	1.06 (0.82–1.38)	1.28 (1.00–1.75)	1.21 (1.01–1.68)	0.05
	HR (95% CI) for women	Ref	1.03 (0.74–1.42)	1.16 (0.84–1.60)	1.09 (0.71–1.40)	0.86 (0.60–1.22)	0.18
Lung	Cases (men/women)	496/946	693/971	845/804	1079/450	1234/405	—
	HR (95% CI)	Ref	1.00 (1.00–1.18)	1.07 (0.98–1.17)	1.16 (1.06–1.26)	1.16 (1.06–1.26)	0.001
Bladder	Cases (men/women)	174/50	236/67	283/55	338/58	380/73	—
	HR (95% CI)	Ref	1.13 (0.95–1.35)	1.14 (0.96–1.36)	1.19 (1.00–1.42)	1.16 (0.98–1.38)	0.26
Kidney	Cases (men/women)	185/93	176/76	197/80	244/65	270/53	—
	HR (95% CI)	Ref	1.19 (0.99–1.36)	1.15 (0.95–1.39)	1.28 (0.99–1.40)	1.18 (0.98–1.43)	0.22
Thyroid	Cases (men/women)	28/98	28/98	34/26	35/37	45/28	—
	HR (95% CI)	Ref	0.88 (0.60–1.21)	0.71 (0.51–1.08)	0.89 (0.64–1.25)	0.93 (0.67–1.31)	0.86
Non-Hodgkin lymphoma	Cases (men/women)	165/180	247/145	253/131	298/138	303/159	—
	HR (95% CI)	Ref	1.10 (0.95–1.28)	1.05 (0.88–1.22)	1.08 (0.93–1.26)	1.03 (0.89–1.20)	0.85
Leukemia	Cases (men/women)	126/79	126/61	155/60	163/49	185/31	—
	HR (95% CI)	Ref	0.83 (0.67–1.01)	1.00 (0.22–1.08)	0.02 (0.67–1.01)	0.88 (0.65–0.90)	0.13
Melanoma	Cases (men/women)	243/158	237/114	295/96	312/100	322/53	—
	HR (95% CI)	Ref	0.81 (0.70–0.94)	0.88 (0.78–1.02)	0.90 (0.78–1.04)	0.82 (0.71–0.96)	0.13
Brain	Cases (men/women)	98/38	85/48	82/31	82/25	75/29	—
	HR (95% CI)	Ref	1.14 (0.88–1.49)	1.01 (0.78–1.32)	0.94 (0.70–1.25)	0.88 (0.65–1.18)	0.15
Myeloma	Cases (men/women)	58/54	57/41	65/31	86/39	115/30	—
	HR (95% CI)	Ref	0.94 (0.71–1.25)	0.91 (0.68–1.22)	0.98 (0.74–1.31)	1.30 (0.99–1.71)	0.01
Prostate	Cases	2,549	3,139	3,312	3,058	4,196	—
	HR (95% CI)	Ref	1.01 (0.97–1.06)	1.01 (0.97–1.07)	1.01 (0.96–1.06)	1.02 (0.97–1.07)	0.60
Female breast	Cases	1,006	1,254	1,192	963	757	—
	HR (95% CI)	Ref	1.04 (0.96–1.13)	0.04 (0.87–1.03)	1.09 (1.00–1.18)	1.03 (0.94–1.12)	0.38
Endometrial	Cases	314	287	233	198	174	—
	HR (95% CI)	Ref	0.83 (0.77–1.12)	0.99 (0.83–1.28)	0.91 (0.75–1.11)	1.02 (0.89–1.23)	0.36
Ovarian	Cases	140	136	109	96	60	—
	HR (95% CI)	Ref	1.14 (0.87–1.50)	1.21 (0.91–1.58)	1.13 (0.85–1.51)	1.23 (0.90–1.63)	0.30
Cervical	Cases	28	25	17	35	24	—
	HR (95% CI)	Ref	0.97 (0.52–1.81)	0.77 (0.39–1.51)	1.05 (0.55–1.98)	1.73 (0.96–3.09)	0.01

Multivariate model includes: age (continuous), sex, education (<8 y or unknown, 8–11 y, 12 y (high school), some college, college graduate), marital status, family history of cancer, race (non-Hispanic white, non-Hispanic black, Hispanic/Asian/Pacific Islander/American Indian/Alaskan native, or unknown), BMI (18.5 to <25, 25 to <30, 30 to <35, ≥35 kg/m²), smoking (never, quit ≥10 y ago, quit 5–9 y ago, quit 1–4 y ago, quit <1 y or currently smoking and smoked ≤20 cigs/d, quit <1 y ago or currently smoking and smoked >20 cigs/d), frequency of vigorous physical activity (never/rarely, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5 or more times/wk), total energy intake (continuous), alcohol intake (none, 0 to <5, 5 to <15, 15 to <30, ≥30 g/d), and fruit and vegetable consumption (quintiles).

[a] Nutrient density energy adjusted.

Ref, reference group.

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Table 3.
Multivariate HRs and 95% CIs (Both Sexes Combined) for Processed Meat Intake and Cancer in the NIH-AARP Diet and Health Study
https://doi.org/10.1371/journal.pmed.0040325.t003

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Surprisingly, both leukemia and melanoma were inversely associated with processed meat intake; the inverse association for leukemia was mainly for lymphocytic leukemia (n = 534; HR = 0.70; 95% CI = 0.52–0.93; p for trend = 0.05) and not myeloid and monocytic leukemia (n = 457; HR = 0.88; 95% CI = 0.64–1.20; p for trend = 0.73). The associations between processed meat intake and cancer risk are summarized in Figure 2, in order of risk magnitude.

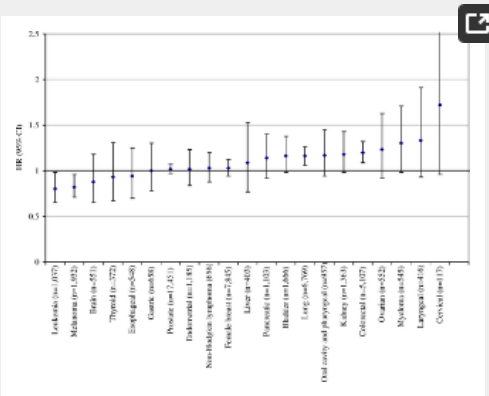

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Figure 2. HRs and 95% CIs for the 5th Versus 1st Quintile of Processed Meat Intake and Cancer Risk for Both Sexes Combined (Except for Sex-Specific Cancers)

https://doi.org/10.1371/journal.pmed.0040325.g002

Sex-specific analyses revealed a positive association for men in the highest quintile of processed meat consumption for pancreatic cancer (HR = 1.31; 95% CI = 1.01–1.68; p for trend = 0.05; p interaction = 0.01); this association was not attenuated by fine control for smoking. Furthermore, women in the highest quintile of processed meat had a borderline statistically significant elevated risk for cervical cancer (HR = 1.72; 95% CI = 0.96–3.09; p for trend = 0.01).

Further analyses did not reveal differences in risk associated with either red or processed meat intake for squamous versus adenocarcinoma of the esophagus, cardia versus non-cardia gastric cancer, and pre- versus post-menopausal breast cancer (unpublished data). However, we observed a suggestion of an elevated risk for advanced prostate cancer (n = 1,782), for both red meat (HR = 1.15; 95% CI = 0.98–1.36; p for trend = 0.21) and processed meat intake (HR = 1.22; 95% CI = 1.05–1.43; p for trend = 0.08), when comparing those in the highest with those in the lowest quintile of intake.

We conducted sensitivity analyses excluding processed meats from the red meat variable to determine whether the risks associated with red and processed meat are independent of each other. The removal of processed meats from the red meat variable reduced the median intake of red meat from 31.4 g/1,000 kcal to 23.6 g/1,000 kcal. The positive associations for red meat and cancer of the liver, esophagus, colorectum, and lung all remain when processed meats were removed from the red meat variable. Furthermore, the inverse association for red meat and endometrial cancer remained after removing processed meats.

In a lag analysis excluding the first two years of follow-up, both the positive and the inverse associations reported in this study remained. A stepwise addition of the covariates to a simple age- and sex-adjusted model showed that the effects of red and processed meat intake on cancer risk were attenuated the most by the addition of the smoking variable to the models. Subanalyses showed that there was no clear gradient in risk for colorectal or lung cancer across categories of education, BMI, physical activity, or alcohol intake. With regard to race, the increased risk observed for red and processed meat and cancer of the colorectum and lung was not evident for blacks, although this ethnicity only represented 3.8 and 4.0 percent of the cases for each cancer site, respectively, and therefore the confidence intervals were wide. The risks for lung cancer associated with both red and processed meat intake were apparent across all categories of smoking.

Discussion

In this large, prospective investigation of red and processed meat intake in relation to cancer risk, we found elevated risks for colorectal and lung cancer with both meat types. Red, but not processed, meat intake was also associated with increased risk for cancer of the esophagus and liver. We observed borderline statistically significant elevated risks for advanced prostate

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cancer with both red and processed meat intake, for laryngeal cancer with red meat, and for bladder cancer and myeloma and with processed meat intake.

The cancer site most consistently associated with meat intake has been the colorectum. A recent meta-analysis of 15 prospective studies published through March, 2006, included approximately 7,500 cases, and reported elevated risks in the highest category of consumption of 28% for red meat and 20% for processed meat [9]. Our study included over 5,000 colorectal cancer cases, and it lends strong support to implicate red and processed meat as risk factors for this malignancy. Consistent with previous studies [9], we observed a stronger positive association for rectal than colon cancer.

The positive associations for both red and processed meat that we report for lung cancer were of similar magnitude to the findings for colorectal cancer. To date, our study includes the largest prospective analysis of meat intake and lung cancer risk. Previous case-control studies have reported elevated risks for lung cancer for those in the highest categories of red meat [17–19], fried red meat [8,19], well-done red meat [17], and processed meat intake [20]. Despite conducting analyses to show that very fine control of smoking history, using a 31-level variable, did not attenuate the lung cancer associations, there remains a potential issue of residual confounding by smoking, because it is such a strong risk factor for this disease.

We found a positive association between red meat intake specifically and cancers of the esophagus and liver, and a borderline significant positive association for laryngeal cancer. The first prospective study of meat intake and esophageal cancer was published recently; that study had only 65 cases and found a positive association for processed meat, but not red meat, with esophageal adenocarcinoma [21]. Our study suggests a threshold effect for red meat intake on esophageal cancer risk, beginning at a low level of intake, with no further increase in risk with higher intakes, as reflected in the *p*-trend ($p = 0.13$), although it is possible that the referent group had a smaller-than-expected cancer incidence by chance. Data on meat intake and cancers of the liver and larynx are limited, and our study is the first prospective investigation to report on these associations. Two case-control studies reported elevated risks for laryngeal cancer for those in the highest intake categories of red meat intake [22,23] and fried beef/veal [24].

In our study, those in the highest quintile of processed meat intake had borderline statistically significant elevated risks for myeloma, a malignancy that has not been well-studied for dietary associations, and bladder cancer. A study of two prospective cohorts combined [25], and one case-control study [26], both found elevated risks of bladder cancer for those in the highest categories of processed meat consumption, but another cohort study found no association [27].

Unexpectedly, we found an inverse association between red meat intake and endometrial cancer; this association was not attenuated by adjustment for known risk factors, such as body mass index or menopausal hormone therapy, or by fine control for smoking, which has been inversely associated with this malignancy [28]. Previous studies have reported null [29,30] or positive relations [31] between red meat and endometrial cancer. We also observed inverse associations between processed meat intake and leukemia and melanoma. In contrast to our findings, childhood leukemia has been positively associated with intake of processed meats in a case-control study [32].

Both red and processed meat intake were positively associated with pancreatic cancer in men, but not women. Red meat has been associated with pancreatic cancer in some [33,34], but not all [35–39] previous cohort studies, as has processed meat in one cohort [34] and several case-control studies [40–44]; although a sex-specific association has not been reported before. Although the association between pancreatic cancer and red or processed meat intake in men was unchanged by fine control for smoking, residual confounding by smoking is still possible.

Our study did not reveal an association between red or processed meat intake and gastric, prostate, or breast cancer, or non-Hodgkin lymphoma. In contrast to the positive relation between both red and processed meat intakes and noncardia gastric cancer in a large cohort in Europe [21], we found no differences in risk according to gastric anatomic subsite. The evidence for a positive association between meat intake and gastric cancer has been more consistent for processed meat than red meat, with elevated risks for processed meat in several case-control [45–52] and cohort studies [53–57], whereas red meat has been positively associated in some [45,46], but not all studies [47,58,59].

Previous studies of meat intake and prostate cancer are conflicting. Some studies have reported null findings [5,60–66], and others suggest positive associations [67–74]. Despite finding no association between red or processed meat intake and overall prostate cancer risk, we observed a suggestion of an elevated risk for advanced prostate cancer with both meat types. If the relation of meat intake to prostate cancer is confined to advanced disease, this could explain some of the inconsistencies in the literature as most previous studies have not specifically addressed advanced prostate cancer.

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With regard to breast cancer, a pooled analysis of eight cohort studies found no association with red meat intake [75]; however, the two most recent prospective studies found positive associations for both red and processed meat [76], specifically for estrogen and progesterone receptor–positive breast cancers in premenopausal women [77]. Although breast cancer risk related to meat intake did not appear to differ by menopausal status in our study, we had very few premenopausal cases (n = 94) and lacked information on hormone receptor status for a large number of cases.

In agreement with our findings, the majority of studies of red meat and non-Hodgkin lymphoma have been null [78–85], although elevated risks were reported in some studies [86–88]; similarly, of nine studies investigating processed meat and non-Hodgkin lymphoma [79–82,84,86–89], only two found a positive association [81,89]. In contrast to our null findings, some case-control studies have reported positive associations for red or processed meat intake and cancer of the oral cavity, pharynx [90,91], kidney [92], ovaries [83], thyroid [83], and brain [93], although data for these cancer sites are limited.

Both red meat, regardless of processing procedure, and processed meat can be linked to carcinogenesis by different mechanisms; for example, they are both sources of saturated fat and iron, which have independently been associated with carcinogenesis. Associations between saturated fat and cancer are likely to be related to energy balance in general, whereas iron is thought to contribute to carcinogenesis specifically by generating free radicals and inducing oxidative stress [94]. Most recently, dietary fat was positively associated with breast cancer [95], and iron intake was positively associated with liver [96] and colorectal cancers [97].

Meat is also a source of several known mutagens, including *N*-nitroso compounds (NOCs), heterocyclic amines (HCAs), and polycyclic aromatic hydrocarbons (PAHs). Exposure to NOCs occurs from endogenous formation, which is directly related to red meat intake [98], and from exogenous exposure from nitrite-preserved meats, for example [1]. Red meat is a large source of readily available heme iron, which has been associated with increased endogenous NOC formation [99]. Human exposure to NOCs and subsequent cancer risk has not been studied extensively; although a Finnish cohort reported an increased risk of colorectal cancer with exogenous exposure to *N*-nitrosodimethylamine (from smoked and salted meats) [6]. In addition, NOC intake and excretion were significantly greater in an area within China considered as high-risk for esophageal cancer [100]. HCAs and PAHs, which are formed during high-temperature cooking of meat [101], dose-dependently generate DNA adducts [3] and tumors in rodents [2,102] in a wide variety of tissues and organs, with similarities between experimental animals and humans [103]. Epidemiologic studies with the capacity to estimate HCA and PAH intake from meat cooking information have found elevated risks of colorectal neoplasia [104–106], squamous cell esophageal cancer [107], as well as cancers of the lung [8], pancreas [4], and prostate [5].

With regard to the stronger relation of red and processed meat to rectal cancer than to colon cancer, there is variation in several characteristics along the large intestine; for example, the average crypt length [108], apoptotic index [109], and propensity to form 0^6-methylguanine adducts[110] (pro-carcinogenic and a marker of NOC exposure) is greater toward the rectum than in the proximal colon. Furthermore, there is variation throughout the colorectum in bacterial enzymes [111], in the fermentation of short chain fatty acids [112], in the expression of metabolic enzymes [113], and in the concentration of fecal matter, in which potential carcinogens are concentrated toward the rectum.

Despite abundant biologic pathways linking meat intake to carcinogenesis at numerous anatomic sites, this is the first comprehensive and prospective analysis of meat intake in relation to a full range of malignancies. A particular strength of this study includes the large size of the cohort, which enabled us to investigate low-incidence cancers that have not previously been prospectively explored. Our findings are strictly generalizable to US whites over 50 y old, but may readily extend to other populations, because it is unlikely that the mechanisms relating meat to carcinogenesis differ quantitatively between our study population and those to whom our results do not strictly apply. An additional strength was that our study encompassed a wide range of reported meat intake, providing adequate statistical power to detect associations. Furthermore, recall bias and reverse causation were minimized by the assessment of diet prior to cancer diagnoses.

Potential limitations of this study include some degree of measurement error associated with the assessment of dietary and lifestyle variables. The FFQ used in this study was compared with two 24-h recall diaries in a subgroup of individuals from this cohort. The energy-adjusted correlation coefficients for protein and saturated fat, the two most relevant macronutrients for meat, were 0.43 and 0.50 for men and women, respectively, for protein, and 0.76 and 0.69 for men and women, respectively, for saturated fat [114]. These correlations compared very favorably to other commonly used FFQs that have correlations ranging from 0.29 to 0.61 in men and from 0.16 to 0.67 in women for protein intake and from 0.64 to 0.76 in men and from 0.59 to 0.76 in women for saturated fat intake [114]. Furthermore, the correlations for red meat assessed from the FFQ compared with two 24-h recall dairies were 0.62 for men and 0.70 for

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women [12]. Although some measurement error remains, the error associated with FFQs tends to result in attenuated risks [115], and we attempted to minimize this error by energy adjustment of the models [116]. With regard to nondietary covariates, reviews and meta-analyses have concluded that self-reported smoking behavior is accurate in most studies as assessed by plasma cotinine levels [117], and the correlations for physical activity assessment in questionnaires similar to that used in our study produce reasonable correlations [118]. Furthermore, self-reported height and weight is a reliable method of estimating BMI.

Some of the observed associations between meat intake and cancer risk in our study may be explained by exposure to HCAs and PAHs from meats cooked well-done by high-temperature cooking techniques; however, we lacked data on detailed cooking preferences from baseline. In addition, because we analyzed cancer incidence at multiple sites, some of our findings may have occurred by chance.

In conclusion, a diet high in red or processed meat was associated with an elevated risk of both colorectal and lung cancer; in addition, red meat was associated with an elevated risk of esophageal and liver cancer. A decrease in the consumption of red and processed meat could reduce the incidence of cancer at multiple sites.

Supporting Information

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Alternative Language Abstract S1. **German Translation of Abstract by M. F. Leitzmann**
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(21 KB DOC)

Text S1. **Meat and Cancer STROBE Statement for Study**
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Author Contributions

AJC had full access to the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors have given full approval to the final manuscript. AJC, MFL, MHG, AS, and RS were involved in the study design, analysis, and interpretation of the data. AJC, MFL, MHG, ARH, AS, and RS were all involved in the critical revision of the manuscript.

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Publications

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U.S. Patent No. 10,039,306

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Attachment 53b

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362 0_ | ‡**a** Vol. 1, issue 1 (Oct. 2004)-
500 __ | ‡**a** Title from cover.
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510 1_ | ‡**9** 1‡**a** MEDLINE‡**b** v1n1, Oct. 2004-
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510 0_ | ‡**a** PMC
510 1_ | ‡**a** Index medicus
530 __ | ‡**a** Also issued online.
650 12 | ‡**a** Medicine
650 22 | ‡**a** Public Health
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856 4_ | ‡**u** https://journals.plos.org/plosmedicine/
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911 __ | ‡**a** Electronic only, print ceased 2014. Confirmed 20160517
935 __ | ‡**a** (DNLM)1231360-nlmdb
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998 __ | ‡**a** FER‡**b** 20130712
999 __ | ‡**a** AUTH
035 __ | ‡**a** (OCoLC)54674092

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Attachment 53c

Medicine MeSH Descriptor Data 2022

Details | Qualifiers | MeSH Tree Structures | Concepts

MeSH Heading	Medicine
Tree Number(s)	H02.403
Unique ID	D008511
RDF Unique Identifier	http://id.nlm.nih.gov/mesh/D008511
Annotation	medicine only as a field, profession or discipline: differentiate from DELIVERY OF HEALTH CARE where the patient is emphasized; very general; it is divided broadly into experimental medicine (= BIOMEDICAL RESEARCH) & CLINICAL MEDICINE, a specialty devoted to the diagnosis & management of human patients; / legislation & jurisprudence = LEGISLATION, MEDICAL or JURISPRUDENCE or FORENSIC MEDICINE; LEGAL MEDICINE see FORENSIC MEDICINE is also available
Scope Note	The art and science of studying, performing research on, preventing, diagnosing, and treating disease, as well as the maintenance of health.
Entry Term(s)	Insurance Medicine Medical Specialities Medical Specialties Medical Specialty Specialities, Medical Specialties, Medical Specialty, Medical
See Also	Economics, Medical Education, Medical History of Medicine Insurance, Health Legislation, Medical
Public MeSH Note	1963; for SPECIALTIES, MEDICAL see SPECIALTIES, MEDICAL 1968-2009
History Note	1963
Entry Combination	economics:Economics, Medical education:Education, Medical ethics:Ethics, Medical

	history:History of Medicine
	legislation & jurisprudence:Legislation, Medical
Date Established	1963/01/01
Date of Entry	1999/01/01
Revision Date	2021/07/06

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Attachment 53d

OCLC 54674092 **No holdings in XXX - 66 other holdings**

Continuing Resources

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362 0 Vol. 1, issue 1 (Oct. 2004)-
500 Title from cover.
530 Also issued online.
588 Latest issue consulted: Vol. 2, issue 1 (Jan. 2005).
650 0 Medicine $v Periodicals.
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Attachment 53e

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

LC control no.: sh2008107665
LCCN Permalink: https://lccn.loc.gov/sh2008107665
HEADING: Medicine Periodicals

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Attachment 53f

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

LC control no.: sh 85108638
LCCN Permalink: https://lccn.loc.gov/sh85108638
HEADING: Public health

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053 _0 |**a** RA
150 __ |**a** Public health
360 __ |**i** subdivision |**a** Health and hygiene |**i** under ethnic groups and classes of persons; and subdivision |**a** Health aspects |**i** under topics and individual wars, e.g. |**a** Recreation areas--Health aspects; World War, 1939-1945--Health aspects
450 __ |**a** Community health
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550 __ |**a** Biosecurity
550 __ |**a** Health literacy
550 __ |**a** Medicine, Preventive
550 __ |**a** National health services
550 __ |**a** Sanitation

670 __ |**a** CDC Foundation web site, viewed March 14, 2019 |**b** (What is public health? Public health is the science of protecting and improving the health of people and their communities. This work is achieved by promoting healthy lifestyles, researching disease and injury prevention, and detecting, preventing and responding to infectious diseases)

906 __ |**t** 1012 |**u** tc03 |**v** 0

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953 __ |**a** xx00 |**b** td04

906 __ |**t** 1307 |**v** 0

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Attachment 54a

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Attachment 54b

OCLC 1090140183 **No holdings in XXX - 87 other holdings**

Books

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245 00 Encyclopedia of analytical science.
250 Third edition / $b editor in chief, Paul J. Worsfold, Alan Townshend, Colin F. Poole, Manuel Miró.
264 1 Amsterdam : $b Elsevier, $c 2019.
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520 The third edition of the Encyclopedia of Analytical Science is a definitive collection of articles covering the latest technologies in application areas such as medicine, environmental science, food science and geology. Meticulously organized, clearly written and fully interdisciplinary, the Encyclopedia of Analytical Science provides foundational knowledge across the scope of modern analytical chemistry, linking fundamental topics with the latest methodologies. Articles will cover three broad areas: analytical techniques (e.g., mass spectrometry, liquid chromatography, atomic spectrometry); areas of application (e.g., forensic, environmental and clinical); and analytes (e.g., arsenic, nucleic acids and polycyclic aromatic hydrocarbons), providing a one-stop resource for analytical scientists.--"Provided by publisher's website'.
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Attachment 55a

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588    ##$aLatest issue consulted: Vol. 116, no. 6 (10 Oct. 2005).
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246 1 $i Beginning in 1995, an additional annual vol. of six bimonthly numbers is issued, with title: $a International journal of cancer. $p Predictive oncology
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546 English and French.
550 Publication of the International Union Against Cancer, <1999-
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Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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MINI-REVIEW | Published: August 1997

Production of D-ribose by fermentation

P. De Wulf & E. J. Vandamme

Applied Microbiology and Biotechnology **48**, 141–148 (1997) | Cite this article

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Abstract

The production of D-ribose by fermentation has received much attention lately, possibly because of the use of this pentose to synthesize antiviral and anticancer drugs. This review briefly outlines the methods that have been used to synthesize D-ribose since it was identified in yeast RNA, and focuses in particular on the latest developments in D-ribose fermentation, which have led to D-ribose yields that exceed 90 g/l. Furthermore, the various transketolase-deficient D-ribose-producing mutants that are used, and the biochemical and genetic rationales applied to select them or to enhance their D-ribose productivities, are dealt with. Attention is also drawn to the unusual pleiotropic characteristics of the mutant strains, as well as to the industrial and academic applications of D-ribose.

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E. J. Vandamme

Additional information

Received: 29 January 1997 / Received revision: 13 March 1997 / Accepted: 15 March 1997

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Keywords

Fermentation | Mutant Strain | Anticancer Drug | Pentose | Late Development

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Structured Fluids as Microreactors for Flavor Formation by the Maillard Reaction

S. Vauthey, Ch. Milo, Ph. Frossard, N. Garti, M. E. Leser, and H. J. Watzke

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Thermal reactions of cysteine/furfural and cysteine/ribose mixtures were studied in model systems to gain more insight into the influence of structured fluids such as L_2 microemulsions and cubic phases on the generation of aroma compounds. Formation of 2-furfurylthiol from cysteine/furfural was particularly efficient in L_2 microemulsions and cubic phases compared to aqueous systems. The reaction led to the formation of two new sulfur compounds, which were identified as 2-(2-furyl)thiazolidine and, tentatively, *N*-(2-mercaptovinyl)-2-(2-furyl)thiazolidine. Similarly, generation of 2-furfurylthiol and 2-methyl-3-furanthiol from cysteine/ribose mixtures was strongly enhanced in structured fluids. The cubic phase was shown to be even more efficient in flavor generation than the L_2 microemulsion. It was denoted "cubic catalyst" or "cubic selective microreactor". The obtained results are interpreted in terms of a surface and curvature control of the reactions defined by the structural properties of the formed surfactant associates.

Keywords: Microemulsion; cubic phase; structured fluids; Maillard reaction; formation of thiols; flavor precursors; 2-furfurylthiol

* Author to whom correspondence should be addressed (telephone +21/785-8026; fax +21/785-8554; e-mail heribert.watzke@rdls.nestle.ch).

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Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

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Myoglobin Chemistry and Meat Color

Annual Review of Food Science and Technology
Vol. 4:79-99 (Volume publication date February 2013)
First published online as a Review in Advance on November 26, 2012
https://doi.org/10.1146/annurev-food-030212-182623

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ABSTRACT
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FACTORS INFLUENCING MEAT COLOR
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INTERACTIONS BETWEEN MYOGLOBIN AND BIOMOLECULES
COLOR DEFECTS IN FRESH MEATS
COLOR DEFECTS IN COOKED MEATS
STRATEGIES TO IMPROVE FRESH MEAT COLOR
STRATEGIES TO IMPROVE COOKED MEAT COLOR
CONCLUSIONS

Case No.: IPR2023-00209
U.S. Patent No. 10,039,306

DISCLOSURE STATEMENT

Abstract

Consumers rely heavily on fresh meat color as an indicator of wholesomeness at the point of sale, whereas cooked color is exploited as an indicator of doneness at the point of consumption. Deviations from the bright cherry-red color of fresh meat lead to product rejection and revenue loss. Myoglobin is the sarcoplasmic heme protein primarily responsible for the meat color, and the chemistry of myoglobin is species specific. The mechanistic interactions between myoglobin and multiple extrinsic and intrinsic factors govern the color of raw as well as cooked meats. The objective of this review is to provide an overview of the current research in meat color and how the findings are applied in the meat industry. Characterizing the fundamental basis of myoglobin's interactions with biomolecules in postmortem skeletal muscles is necessary to interpret the chemistry of meat color phenomena and to engineer innovative processing strategies to minimize meat discoloration–induced revenue loss to the agricultural economy.

Keywords

heme protein, oxidation, pigment, cooked color, color defects

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Case No.: IPR2023-00209
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Journal of Food Science / Volume 53, Issue 2 / p. 658-658

Identification of 2-Methyl-3-Furanthiol in the Steam Distillate from Canned Tuna Fish

D.ONALD A. WITHYCOMBE, C.YNTHIA J. MUSSINAN

First published: March 1988
https://doi.org/10.1111/j.1365-2621.1988.tb07781.x
Citations: 34

About

ABSTRACT

The volatile components of canned tuna fish were isolated by steam distillation followed by solvent extraction. Analysis of the extract using a sulfur-specific detector and coupled gas chromatography -mass spectrometry permitted the identification of 2-methyl-3-furanthiol, a compound with an intense beef extract aroma. Mass spectral data are presented.

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Quantification of 2-Methyl-3-furanthiol, 2-Furfurylthiol, 3-Mercapto-2-pentanone, and 2-Mercapto-3-pentanone in Heated Meat

Roland Kerscher and Werner Grosch

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Abstract

A stable isotope dilution assay was developed for quantification of the potent odorants 2-methyl-3-furanthiol (MFT), 2-furfurylthiol (FFT), 2-mercapto-3-pentanone (2M3P), and 3-mercapto-2-pentanone (3M2P) in heated meat. The volatiles of meat were extracted with dichloromethane, which was spiked with definite amounts of stable isotopomers of MFT, FFT, and 3M2P. The analytes and their labeled standards were enriched by reaction with *p*-hydroxymercuribenzoic acid, and after liberation, the thiols were assayed by dynamic headspace gas chromatography in combination with mass spectrometry. The following amounts (micrograms per kilogram) were found in meat samples boiled for 45 min: (beef) MFT, 7–28, FFT, 13–42, 3M2P, 55–73, 2M3P, 20–44; (pork) MFT, 6–9, FFT, 8–10, 3M2P, 66–117, 2M3P, 11–14; (lamb) MFT, 5–11, FFT, 9–14, 3M2P, 30, 2M3P, 10. Chicken contained per kilogram 4.5 µg of MFT, 2.4 µg of FFT, 100 µg of 3M2P, and 13 µg of 2M3P after a boiling period of 60 min.

Keywords: Isotope dilution assay; 2-methyl-3-furanthiol; 2-furfurylthiol; 3-mercapto-2-pentanone; 2-mercapto-3-pentanone; beef; chicken; pork; lamb; flavor

* Author to whom correspondence should be addressed (telephone +49-89-289 141 70; fax +49-89-289 141 83; e-mail Werner.Grosch@lrz.tu-muenchen.de).

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Studies on the Formation and Stability of the Roast-Flavor Compound 2-Acetyl-2-thiazoline

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2-(1-Hydroxyethyl)-4,5-dihydrothiazole (HDT) was identified by ^{1}H- and ^{13}C-NMR measurements as the main volatile product formed by storage of an aqueous solution of cysteamine and methylglyoxal at low temperatures (6 °C). The structure of HDT was confirmed by synthesis. Systematic studies on the thermal stability of the HDT under different conditions either in aqueous solution or during high-resolution gas chromatography revealed that HDT is an important intermediate in the formation of the intensely roasty smelling food flavor compound 2-acetyl-2-thiazoline (AT). Model studies showed that more than 10% of the precursor HDT was converted into AT simply by heating for 10 min in water. The activation energy of this reaction was 57.4 kJ/mol. Experiments on the thermal stability of AT itself revealed that heating in aqueous solution also led to a degradation of AT, whereas heating in an oil significantly stabilized the flavor compound.

Keywords: *2-(1-Hydroxyethyl)-4,5-dihydrothiazole; 2-acetyl-2-thiazoline; flavor precursor; stable isotope dilution analysis*

INTRODUCTION

2-Acetyl-2-thiazoline (AT) has been reported for the first time among food flavors as a volatile constituent of beef broth (Tonsbeek et al., 1971). The compound was later identified in roast beef (Hartmann et al., 1983) and, also, overpasteurized beer (Tressl et al., 1983).

Using aroma extract dilution analysis (AEDA), the flavor compound, eliciting an attractive roasty, popcorn-like odor, has recently been established as a key contributor to several processed meat products, such as chicken broth (Gasser and Grosch, 1990), roast beef (Cerny and Grosch, 1993), stewed beef (Guth and Grosch, 1993), boiled trouts (Milo and Grosch, 1993), and roasted white sesame seeds (Schieberle, 1993). Furthermore, AT was detected among the odor-active volatiles of stewed beef juice (Guth and Grosch, 1994).

Volatile fractions generated by reacting cysteine with carbohydrates exhibit flavors resembling processed beef meat. Investigations on the chemical composition of such fractions have led to the identification of AT among the numerous volatiles generated from such precursor mixtures (Sakaguchi and Shibamoto, 1978; Silwar, 1992). Recent studies applying AEDA on the volatiles formed by the reaction of ribose/cysteine (Hofmann and Schieberle, 1995) have revealed that AT in fact contributes significantly to the roasty odors, and it was concluded that AT is a key odorant of such Maillard-type reaction flavors containing cysteine.

Hayashi and Shibamoto (1985) have reported that AT is formed in relatively high yields by reacting 1-mercapto-2-aminoethane (cysteamine) with 2-oxopropanal (methylglyoxal). This result suggested both compounds are important intermediates in AT formation from cysteine and carbohydrates. On the basis of the fact that at lower temperatures the 2-acetylthiazolidine was predominantly formed, it has been assumed (Hayashi and Shibamoto, 1985) that the flavor compound may be formed by oxidation of this intermediate.

Precursors generating meat-like odorants upon heating are of special interest since they would offer the possibility to enhance the desired flavor *in situ* during cooking.

The following investigation was undertaken to gain a more detailed insight into the intermediates and reaction mechanisms governing the formation of AT from cysteine and carbohydrates and, furthermore, to study the thermal stability of AT.

MATERIALS AND METHODS

Chemicals. 1-Mercapto-2-aminoethane hydrochloride (cysteamine hydrochloride), 2-oxopropanal (methylglyoxal; 40% in water), hydroxypropanoic acid nitrile, and lithium aluminum hydride were obtained commercially (Aldrich, Steinheim, Germany).

Synthesis. *2-(1-Hydroxyethyl)-4,5-dihydrothiazole (HDT).* A mixture of cysteamine hydrochloride (12 mmol), 2-hydroxypropanoic acid nitrile (4 mmol), and ammonium acetate (1 g) in methanol (4 mL) was stirred for 32 h at room temperature. After the addition of water (10 mL), the HDT formed was extracted with diethyl ether (total volume 50 mL), and an aliquot was purified by flash chromatography as detailed in the following: The stationary phase (Bakerbond Diol; 40 μm) was filled 15 cm high into a glass column (35.9 × 1.9 cm; J. T. Baker No. 7022-01) which was equipped with a reservoir (250 mL) and a pressure regulator. The material was then equilibrated by flushing with *n*-pentane/diethyl ether (100 mL, 8 + 2 by vol) under a slight pressure of nitrogen. After application of the concentrated reaction mixture (10 mL) onto the column, elution (6 mL/min) was performed using the following *n*-pentane/diethyl ether mixtures: 180 mL/8 + 2 by vol (fraction A), 180 mL/7 + 3 by vol (fraction B), 150 mL/6 + 4 by vol (fraction C1), 30 mL/6 + 4 by vol (fraction C2), 30 mL/1 + 1 by vol (fraction D1) and another 150 mL/1 + 1 by vol (fraction D2). The 2-(1-hydroxyethyl)-4,5-dihydrothiazole, eluting in fractions C2–D2 (overall yield 61%) was characterized by ^{1}H-NMR (cf. Tables 1 and 2) and mass spectral measurements.

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