WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC Handbooks of Cancer Prevention

Non-steroidal Anti-inflammatory Drugs

Volume 1

This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of Cancer Preventive Agents,
which met in Lyon,

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International Agency For Research On Cancer

The International Agency for Research on Cancer (IARC) was established in 1965 by the World Health Assembly, as an independently financed organization within the framework of the World Health Organization. The headquarters of the Agency are in Lyon, France.

The Agency conducts a programme of research concentrating particularly on the epidemiology of cancer and the study of potential carcinogens in the human environment. Its field studies are supplemented by biological and chemical research carried out in the Agency's laboratories in Lyon and, through collaborative research agreements, in national research institutions in many countries. The Agency also conducts a programme for the education and training of personnel for cancer research.

The publications of the Agency contribute to the dissemination of authoritative information on different aspects of cancer research. A complete list is printed at the back of this book. Information about IARC publications, and how to order them, is also available via the Internet at: **http://www.iarc.fr/**

Note to the Reader

Anyone who is aware of published data that may influence any consideration in these *Handbooks* is encouraged to make the information available to the Unit of Chemoprevention, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France

Although all efforts are made to prepare the *Handbooks* as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Unit of Chemoprevention, so that corrections can be reported in future volumes.

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Preamble to the IARC *Handbooks of Cancer Prevention*

The prevention of cancer is one of the key objectives of the International Agency for Research on Cancer (IARC). This may be achieved by avoiding exposures to known cancer-causing agents, by increasing host defence through immunization or chemoprevention, or by modifying lifestyle. The aim of the *IARC Monographs* programme is to evaluate carcinogenic risks of human exposure to chemical, physical and biological agents, providing a scientific basis for national or international decisions on avoidance of exposures. The aim of the series of the *IARC Handbooks of Cancer Prevention* is to evaluate scientific information on agents and interventions that may reduce the incidence of or mortality from cancer. This preamble is divided into two parts. The first addresses the general scope, objectives and structure of the *Handbooks*. The second describes the procedures for evaluating chemopreventive agents.

Part One

Scope

Prevention strategies embrace chemical, immunological, dietary and behavioural interventions that may retard, block or reverse carcinogenic processes or reduce underlying risk factors. The term 'chemoprevention' is used to refer to interventions with pharmaceuticals, vitamins, minerals and other chemicals to reduce cancer incidence. The *IARC Handbooks* address the efficacy, safety and mechanisms of cancer-preventive strategies and the adequacy of the available data, including those on timing, dose, duration and indications for use.

Prevention strategies can be applied across a continuum of: (1) the general population; (2) subgroups with particular predisposing host or environmental risk factors, including genetic susceptibility to cancer; (3) persons with precancerous lesions; and (4) cancer patients at risk for second primary tumours. Use of the same strategies or agents in the treatment of cancer patients to control the growth, metastasis and recurrence of tumours is considered to be patient management, not prevention, although data from clinical trials may be relevant when making a *Handbooks* evaluation.

Objective

The objective of the *Handbooks* programme is the preparation of critical reviews and evaluations of evidence on the cancer-preventive and other relevant properties of a wide range of potential cancer-preventive agents and strategies by international working groups of experts. The resulting *Handbooks* may also indicate where additional research is needed.

The *Handbooks* may assist national and international authorities in devising programmes of health promotion and cancer prevention and in making benefit–risk assessments. The evaluations of IARC working groups are scientific judgements about the available evidence for cancer-preventive efficacy and safety. No recommendation is given with regard to national and international regulation or legislation, which are the responsibility of individual governments and/or other international authorities. No recommendations for specific research trials are made.

IARC Working Groups

Reviews and evaluations are formulated by international working groups of experts convened by the IARC. The tasks of each group are: (1) to ascertain that all appropriate data have been collected; (2) to select the data relevant for the evaluation on the basis of scientific merit; (3) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (4) to evaluate the significance of the available data from human studies and experimental models on cancer-preventive activity, carcinogenicity and other beneficial and adverse effects; and (5) to evaluate data relevant to the understanding of mechanisms of action.

Working Group participants who contributed to the considerations and evaluations within a particular *Handbook* are listed, with their addresses, at the beginning of each publication. Each participant serves as an individual scientist and not as a representative of any organization, government or industry. In addition, scientists nominated by national and international agencies, industrial associations and consumer and/or environmental organizations may be invited as observers. IARC staff involved in the preparation of the *Handbooks* are listed.

Working procedures

Approximately 13 months before a working group meets, the topics of the *Handbook* are announced, and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant clinical, experimental and human data are collected by the IARC from all available sources of published information. Representatives from producer or consumer associations may assist in the preparation of sections on production and use as appropriate.

About eight months before the meeting, the material collected is sent to meeting participants to prepare sections for the first drafts of the *Handbooks*. These are then compiled by IARC staff and sent, before the meeting, to all participants of the Working Group for review. There is an opportunity to return the compiled specialized sections of the draft to the experts, inviting preliminary comments, before the complete first draft document is distributed to all members of the Working Group.

Data for *Handbooks*

The *Handbooks* do not necessarily cite all of the literature on the agent or strategy being evaluated. Only those data considered by the Working Group to be relevant to making the evaluation are included. In principle, meeting abstracts and other reports that do not provide sufficient detail upon which to assess their quality should be avoided.

With regard to data from toxicological, epidemiological and experimental studies and from clinical trials, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed by the Working Group. In certain instances, government agency reports that have undergone peer review and are widely available are considered. Exceptions may be made on an ad-hoc basis to include unpublished reports that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation. In the sections on chemical and physical properties, on production, on use, on analysis and on human exposure, unpublished sources of information may be used.

Criteria for selection of topics for evaluation

Agents, classes of agents and interventions to be evaluated in the *Handbooks* are selected on the basis of one or more of the following criteria.

- The available evidence suggests potential for significantly reducing the incidence of cancers.
- There is a substantial body of human, experimental, clinical and/or mechanistic data suitable for evaluation.
- The agent is in widespread use and of putative protective value, but of uncertain efficacy and safety.
- The agent shows exceptional promise in experimental studies but has not been used in humans.
- The agent is available for further studies of human use.

Part Two

Evaluation of cancer-preventive agents

A wide range of findings must be taken into account before a particular agent can be recognized as preventing cancer. On the basis of experience from the *IARC Monographs* programme, a systematized approach to data presentation is adopted for *Handbooks* evaluations.

1. Chemical and physical characteristics of the agent

The Chemical Abstracts Services Registry Number, the latest Chemical Abstracts Primary Name, the IUPAC Systematic Name and other definitive information (such as genus and species of plants) are given as appropriate. Information on chemical and physical properties and, in particular, data relevant to identification, occurrence and biological activity are included. A description of technical products of chemicals includes trade names,

Outline of data presentation scheme for evaluating chemopreventive agents

1. **Chemical and physical characteristics**
2. **Occurrence, production, use, analysis and human exposure**
   - 2.1 Occurrence
   - 2.2 Production
   - 2.3 Use
   - 2.4 Analysis
   - 2.5 Human exposure
3. **Metabolism, kinetics and genetic variation**
   - 3.1 Human studies
   - 3.2 Experimental models
   - 3.3 Genetic variation
4. **Cancer-preventive effects**
   - 4.1 Human studies
   - 4.2 Experimental models
     - 4.2.1 Experimental animals
     - 4.2.2 *In vitro* models
   - 4.3 Mechanisms of chemoprevention
5. **Other beneficial effects**
6. **Carcinogenicity**
   - 6.1 Humans
   - 6.2 Experimental animals
7. **Other toxic effects**
   - 7.1 Adverse effects
     - 7.1.1 Humans
     - 7.1.2 Experimental animals
   - 7.2 Genetic and related effects
     - 7.2.1 Humans
     - 7.2.2 Experimental models
8. **Summary of data**
   - 8.1 Chemistry, occurrence and human exposure
   - 8.2 Metabolism and kinetics
   - 8.3 Cancer-preventive effects
     - 8.3.1 Humans
     - 8.3.2 Experimental animals
     - 8.3.3 Mechanism of action
   - 8.4 Other beneficial effects
   - 8.5 Carcinogenicity
     - 8.5.1 Humans
     - 8.5.2 Experimental animals
   - 8.6 Toxic effects
     - 8.6.1 Humans
     - 8.6.2 Experimental animals
9. **Recommendations for research**
10. **Evaluation**
    - 10.1 Cancer-preventive activity
      - 10.1.1 Humans
      - 10.1.2 Experimental animals
    - 10.2 Overall evaluation

relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

2. Occurrence, production, use, analysis and human exposure

2.1 Occurrence

Information on the occurrence of an agent or mixture in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are included. For mixtures, information is given about all agents present.

2.2 Production

The dates of first synthesis and of first commercial production of a chemical or mixture are provided; for agents that do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human use of, or exposure to, the agent could have occurred. The dates of first reported occurrence of an exposure are also provided. In addition, methods of

synthesis used in past and present commercial production and methods of production that may give rise to different impurities are described.

2.3 Use

Data on production, international trade and uses and applications are obtained for representative regions. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic applications does not necessarily represent current practice, nor does it imply judgement as to their therapeutic efficacy.

2.4 Analysis

An overview of current methods of analysis or detection is presented. Methods for monitoring human exposure are also given, when available.

2.5 Human exposure

Human uses of, or exposure to, the agent are described. If an agent is used as a prescribed or over-the-counter pharmaceutical product, then the type of person receiving the product in terms of health status, age, sex and medical condition being treated are described. For nonpharmaceutical agents, particularly those taken because of cultural traditions, the characteristics of use or exposure and the relevant populations are described. In all cases, quantitative data, such as dose-response data, are considered to be of special importance.

3. Metabolism, kinetics and genetic variation

In evaluating the potential utility of a suspected chemopreventive agent or strategy, a number of different properties, in addition to direct effects upon cancer incidence, are described and weighed. Furthermore, as many of the data leading to an evaluation are expected to come from studies in experimental animals, information that facilitates interspecies extrapolation is particularly important; this includes metabolic, kinetic and genetic data. Whenever possible, quantitative data, including information on dose, duration and potency, are considered.

Information is given on absorption, distribution (including placental transfer), metabolism and excretion in humans and experimental animals. Kinetic properties within the target species may affect the interpretation and extrapolation of dose–response relationships, such as blood concentrations, protein binding, tissue concentrations, plasma half-lives and elimination rates. Comparative information on the relationship between use or exposure and the dose that reaches the target site may be of particular importance for extrapolation between species. Studies that indicate the metabolic pathways and fate of the agent in humans and experimental animals are summarized, and data on humans and experimental animals are compared when possible. Observations are made on interindividual variations and relevant metabolic polymorphisms. Data indicating long-term accumulation in human tissues are included. Physiologically based pharmacokinetic models and their parameter values are relevant and are included whenever they are available. Information on the fate of the compound within tissues and cells (transport, role of cellular receptors, compartmentalization, binding to macromolecules) is given.

Genotyping will be used increasingly, not only to identify subpopulations at increased or decreased risk for cancers but also to characterize variation in the biotransformation of, and responses to, chemopreventive and chemotherapeutic agents.

This subsection can include effects of the compound on gene expression, enzyme induction or inhibition, or pro-oxidant status, when such data are not described elsewhere. It covers data obtained in humans and experimental animals, with particular attention to effects of long-term use and exposure.

4. Cancer-preventive effects

4.1 Human studies

Types of studies considered. Human data are derived from experimental and non-experimental study designs and are focused on cancer, precancer or intermediate biological end-points. The experimental designs include randomized controlled trials and short-term experimental studies; non-experimental designs include cohort, case–control and cross-sectional studies.

Cohort and case–control studies relate individual use of, or exposure to, the agents under study to the occurrence or prevention of cancer in individuals and provide an estimate of relative risk (ratio of incidence or mortality in those exposed to incidence or mortality in those not exposed) as the main measure of association. Cohort and case–control studies follow an observational approach, in which the use of, or exposure to, the agent is not controlled by the investigator.

Intervention studies are experimental in design — that is, the use of, or exposure to, the agent is assigned by the investigator. The intervention study or clinical trial is the design that can provide the strongest and most direct evidence of a protective or preventive effect; however, for practical and ethical reasons, such studies are limited to observation of the effects among specifically defined study subjects of interventions of 10 years or fewer, which is relatively short when compared with the overall lifespan.

Intervention studies may be undertaken in individuals or communities and may or may not involve randomization to use or exposure. The differences between these designs is important in relation to analytical methods and interpretation of findings.

In addition, information can be obtained from reports of correlation (ecological) studies and case series; however, limitations inherent in these approaches usually mean that such studies carry limited weight in the evaluation of a preventive effect.

Quality of studies considered. The *Handbooks* are not intended to summarize all published studies. It is important that the Working Group consider the following aspects: (1) the relevance of the study; (2) the appropriateness of the design and analysis to the question being asked; (3) the adequacy and completeness of the presentation of the data; and (4) the degree to which chance, bias and confounding may have affected the results.

Studies that are judged to be inadequate or irrelevant to the evaluation are generally omitted. They may be mentioned briefly, particularly when the information is considered to be a useful supplement to that in other reports or when it is the only data available. Their inclusion does not imply acceptance of the adequacy of the study design, nor of the analysis and interpretation of the results, and their limitations are outlined.

Assessment of the cancer-preventive effect at different doses and durations. The Working Group gives special attention to quantitative assessment of the preventive effect of the agent under study, by assessing data from studies at different doses. The Working Group also addresses issues of timing and duration of use or exposure. Such quantitative assessment is important to clarify the circumstances under which a preventive effect can be achieved, as well as the dose at which a toxic effect has been shown.

Criteria for a cancer-preventive effect. After summarizing and assessing the individual studies, the Working Group makes a judgement concerning the evidence that the agent in question prevents cancer in humans. In making their judgement, the Working Group considers several criteria for each relevant cancer site.

Evidence of protection derived from intervention studies of good quality is particularly informative. Evidence of a substantial and significant reduction in risk, including a dose–response relationship, is more likely to indicate a real effect. Nevertheless, a small effect, or an effect without a dose–response relationship, does not imply lack of real benefit and may be important for public health if the cancer is common.

Evidence is frequently available from different types of studies and is evaluated as a whole. Findings that are replicated in several studies of the same design or using different approaches are more likely to provide evidence of a true protective effect than isolated observations from single studies.

The Working Group evaluates possible explanations for inconsistencies across studies, including differences in use of, or exposure to, the agent, differences in the underlying risk for cancer and metabolism and genetic differences in the population.

The results of studies judged to be of high quality are given more weight. Note is taken of both the applicability of preventive action to several cancers and of possible differences in activity, including contradictory findings, across cancer sites.

Data from human studies (as well as from experimental models) that suggest plausible mechanisms

for a cancer-preventive effect are important in assessing the overall evidence.

The Working Group may also determine whether, on aggregate, the evidence from human studies is consistent with a lack of preventive effect.

4.2 Experimental models

4.2.1 Experimental animals

Animal models are an important component of chemopreventive research. They provide a means of identifying effective compounds, of carrying out fundamental investigations into their mechanisms of action, of determining how they can be used optimally, of evaluating toxicity and, ultimately, of providing an information base for developing intervention trials in humans. Models that permit evaluation of the effects of chemopreventive agents on the occurrence of cancer in most major organ sites are available. Major groups of animal models include: those in which carcinogenesis is produced by the administration of chemical or physical carcinogens; those involving genetically engineered animals; and those in which tumours develop spontaneously. Most chemopreventive agents investigated in such studies can be placed into one of three categories: compounds that prevent molecules from reaching or reacting with critical target sites (blocking agents); compounds that decrease the sensitivity of target tissues to carcinogenic stimuli; and compounds that prevent evolution of the neoplastic process (suppressing agents). There is increasing interest in the use of combinations of agents as a means of improving efficacy and minimizing toxicity. Animal models are useful in evaluating such combinations. The development of optimal strategies for human intervention trials can be facilitated by the use of animal models that mimic the neoplastic process in humans.

Specific factors to be considered in such experiments are: (1) the temporal requirements of administration of the chemopreventive agents; (2) dose–response effects; (3) the site-specificity of a chemopreventive action; and (4) the number and structural diversity of carcinogens whose action can be reduced by the agent being evaluated. Other types of studies include experiments in which the end-point is not cancer but a defined preneoplastic lesion or tumour-related, inter-mediate biomarker. An important variable in the evaluation of the cancer-preventive response is the time and the duration of the administration of the chemopreventive agent in relation to any carcinogenic treatment, or in transgenic or other experimental models in which no carcinogen is administered. Furthermore, concurrent administration of a chemopreventive agent may result in a decreased incidence of tumours in a given organ and an increase in another organ of the same animal. Thus, in these experiments it is important that multiple organs be examined.

For all these studies, the nature and extent of impurities or contaminants present in the chemopreventive agent or agents being evaluated are given when available. For experimental studies of mixtures, consideration is given to the possibility of changes in the physicochemical properties of the test substance during collection, storage, extraction, concentration and delivery. Chemical and toxicological interactions of the components of mixtures may result in nonlinear dose–response relationships.

As certain components of commonly used diets for experimental animals are themselves known to have chemopreventive activity, particular consideration should be given to the interaction between the diet and the apparent effect of the agent being studied. Likewise, restriction of diet may be important. The appropriateness of the diet given relative to the composition of human diets may be commented on by the Working Group.

Qualitative aspects. An assessment of the experimental prevention of cancer involves several considerations of qualitative importance, including: (1) the experimental conditions under which the test was performed (route and schedule of exposure, species, strain, sex and age of animals studied, duration of the exposure, and duration of the study); (2) the consistency of the results, for example across species and target organ(s); (3) the stage or stages of the neoplastic process, from preneoplastic lesions and benign tumours to malignant neoplasms, studied and (4) the possible role of modifying factors.

Considerations of importance to the Working Group in the interpretation and evaluation of a particular study include: (1) how clearly the agent was defined and, in the case of mixtures, how

adequately the sample composition was reported; (2) the composition of the diet and the stability of the agent in the diet; (3) whether the source, strain and quality of the animals was reported; (4) whether the dose and schedule of treatment with the known carcinogen were appropriate in assays of combined treatment; (5) whether the doses of the chemopreventive agent were adequately monitored; (6) whether the agent(s) was absorbed, as shown by blood concentrations; (7) whether the survival of treated animals was similar to that of controls; (8) whether the body and organ weights of treated animals were similar to those of controls; (9) whether there were adequate numbers of animals, of appropriate age, per group; (10) whether animals of each sex were used, if appropriate; (11) whether animals were allocated randomly to groups; (12) whether appropriate respective controls were used; (13) whether the duration of the experiment was adequate; (14) whether there was adequate statistical analysis; and (15) whether the data were adequately reported. If available, recent data on the incidence of specific tumours in historical controls, as well as in concurrent controls, are taken into account in the evaluation of tumour response. The observation of effects on the occurrence of lesions presumed to be preneoplastic or the emergence of benign or malignant tumours may in certain instances aid in assessing the mode of action of the presumed chemopreventive agent. Particular attention is given to assessing the reversibility of these lesions and their predictive value in relation to cancer development.

Quantitative aspects. The probability that tumours will occur may depend on the species, sex, strain and age of the animals, the dose of carcinogen (if any), the dose of the agent, and the route and duration of exposure. A decreased incidence and/or decreased multiplicity of neoplasms in adequately designed studies provides evidence of a chemopreventive effect. A dose-related decrease in incidence and/or multiplicity further strengthens this association.

Statistical analysis. Major factors considered in the statistical analysis by the Working Group include the adequacy of the data for each treatment group: (1) the initial and final effective numbers of animals studied and the survival rate; (2) body weights; and (3) tumour incidence and multiplicity. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose. In particular, the statistical methods should be appropriate for the characteristics of the expected data distribution and should account for interactions in multifactorial studies. Consideration is given as to whether the appropriate adjustments were made for differences in survival.

4.2.2 *In-vitro models*

Cell systems *in vitro* contribute to the early identification of potential chemopreventive agents and to elucidation of mechanistic aspects. A number of assays in prokaryotic and eukaryotic systems are used for this purpose. Evaluation of the results of such assays includes consideration of: (1) the nature of the cell type used; (2) whether primary cell cultures or cell lines (tumorigenic or nontumorigenic) were studied; (3) the appropriateness of controls; (4) whether toxic effects were considered in the outcome; (5) whether the data were appropriately summated and analysed; (6) whether appropriate quality controls were used; (7) whether appropriate concentration ranges were used; (8) whether adequate numbers of independent measurements were made per group; and (9) the relevance of the end-points, including inhibition of mutagenesis, morphological transformation, anchorage-independent growth, cell–cell communication, calcium tolerance and differentiation.

4.3 Mechanisms of chemoprevention

Data on mechanisms can be derived from both human and experimental systems. For a rational implementation of chemopreventive measures, it is essential not only to assess protective end-points but also to understand the mechanisms by which the agents exert their anticarcinogenic action. Information on the mechanisms of chemopreventive agents can be inferred from relationships between chemical structure and biological activity, from analysis of interactions between agents and specific molecular targets, from studies of specific end-points *in vitro*, from studies of the inhibition of tumorigenesis *in vivo* and the efficacy

of modulating intermediate biomarkers, and from human studies. Therefore, the Working Group takes account of mechanistic data in making the final evaluation of chemoprevention.

Several classifications of mechanisms have been proposed, as have several systems for evaluating them. Chemopreventive agents may act at several distinct levels. Their action may be: (1) extracellular, for example, inhibiting the uptake or endogenous formation of carcinogens, or forming complexes with, diluting and/or deactivating carcinogens; (2) intracellular, for example, trapping carcinogens in non-target cells, modifying transmembrane transport, modulating metabolism, blocking reactive molecules, inhibiting cell replication or modulating gene expression or DNA metabolism; or (3) at the level of the cell, tissue or organism, for example, affecting cell differentiation, intercellular communication, proteases, signal transduction, growth factors, cell adhesion molecules, angiogenesis, interactions with the extracellular matrix, hormonal status and the immune system.

Many chemopreventive agents are known or suspected to act by several mechanisms, which may operate in a coordinated manner and allow them a broader spectrum of anticarcinogenic activity. Therefore, multiple mechanisms of action are taken into account in the evaluation of chemoprevention.

Beneficial interactions, generally resulting from exposure to inhibitors that work through complementary mechanisms, are exploited in combined chemoprevention. Because organisms are naturally exposed not only to mixtures of carcinogenic agents but also to mixtures of protective agents, it is also important to understand the mechanisms of interactions between inhibitors.

5. Other beneficial effects

This section contains mainly background information on preventive activity. Use is described in Section 2.3. An expanded description is given, when appropriate, of the efficacy of the agent in the maintenance of a normal healthy state and the treatment of particular diseases. Information on the mechanisms involved in these activities is described. Reviews, rather than individual studies, may be cited as references.

The physiological functions of agents such as vitamins and micronutrients can be described briefly, with reference to reviews. Data on the therapeutic effects of drugs approved for clinical use are summarized.

6. Carcinogenicity

Some agents may have both carcinogenic and anticarcinogenic activities. If the agent has been evaluated within the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, that evaluation is accepted, unless significant new data have appeared that may lead the Working Group to reconsider the evidence. When a re-evaluation is necessary or when no carcinogenic evaluation has been made, the procedures described in the Preamble to the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* are adopted as guidelines.

7. Other toxic effects

Toxic effects are of particular importance in the case of agents that may be used widely over long periods in healthy populations. Data are given on acute and chronic toxic effects, such as organ toxicity, increased cell proliferation, immunotoxicity and adverse endocrine effects. If the agent occurs naturally or has been in clinical use previously, the doses and durations used in chemopreventive trials are compared with intakes from the diet, in the case of vitamins, and previous clinical exposure, in the case of drugs already approved for human use. When extensive data are available, only summaries are presented; if adequate reviews are available, reference may be made to these. If there are no relevant reviews, the evaluation is made on the basis of the same criteria as are applied to epidemiological studies of cancer. Differences in response as a consequence of species, sex, age and genetic variability are presented when the information is available.

Data demonstrating the presence or absence of adverse effects in humans are included; equally, lack of data on specific adverse effects is stated clearly.

Findings in humans and experimental animals are presented sequentially under the headings 'Toxic and other adverse effects' and 'Genetic and related effects'.

The section 'Toxic and other adverse effects' includes information on immunotoxicity, neuro-

toxicity, cardiotoxicity, haematological effects and toxicity to other target organs. Specific case reports in humans and any previous clinical data are noted. Other biochemical effects thought to be relevant to adverse effects are mentioned. The reproductive and developmental effects described include effects on fertility, teratogenicity, fetotoxicity and embryotoxicity. Information from nonmammalian systems and *in vitro* are presented only if they have clear mechanistic significance.

The section 'Genetic and related effects' includes results from studies in mammalian and nonmammalian systems *in vivo* and *in vitro*. Information on whether DNA damage occurs via direct interaction with the agent or via indirect mechanisms (e.g. generation of free radicals) is included, as is information on other genetic effects such as mutation, recombination, chromosomal damage, aneuploidy, cell immortalization and transformation, and effects on cell–cell communication. The presence and toxicological significance of cellular receptors for the chemopreventive agent are described.

The adequacy of epidemiological studies of toxic effects, including reproductive outcomes and genetic and related effects in humans, is evaluated by the same criteria as are applied to epidemiological studies of cancer. For each of these studies, the adequacy of the reporting of sample characterization is considered and, where necessary, commented upon. The available data are interpreted critically according to the end-points used. The doses and concentrations used are given, and, for *in vitro* experiments, mention is made of whether the presence of an exogenous metabolic system affected the observations. For in vivo studies, the route of administration and the formulation in which the agent was administered are included. The dosing regimens, including the duration of treatment, are also given. Genetic data are given as listings of test systems, data and references; bar graphs (activity profiles) and corresponding summary tables with detailed information on the preparation of genetic activity profiles are given in appendices. Genetic and other activity in humans and experimental mammals is regarded as being of greater relevance than that in other organisms. The in-vitro experiments providing these data must be carefully evaluated, since there are many trivial reasons why a response to one agent may be modified by the addition of another.

Structure–activity relationships that may be relevant to the evaluation of the toxicity of an agent are described.

Studies on the interaction of the suspected chemopreventive agent with toxic and subtoxic doses of other substances are described, the objective being to determine whether there is inhibition or enhancement, additivity, synergism or potentiation of toxic effects over an extended dose range.

Biochemical investigations that may have a bearing on the mechanisms of toxicity and chemoprevention are described. These are carefully evaluated for their relevance and the appropriateness of the results.

8. Summary of data

In this section, the relevant human and experimental data are summarized. Inadequate studies are generally not summarized; such studies, if cited, are identified in the preceding text.

8.1 Chemistry, occurrence and human exposure

Human exposure to an agent is summarized on the basis of elements that may include production, use, occurrence in the environment and determinations in human tissues and body fluids. Quantitative data are summarized when available.

8.2 Metabolism and kinetics

Data on metabolism and kinetics in humans and in experimental animals are given when these are considered relevant to the possible mechanisms of chemoprotective, carcinogenic and toxic activity.

8.3 Cancer-preventive effects

8.3.1 Humans

The results of relevant studies are summarized, including case reports and correlation studies when considered important.

8.3.2 Experimental animals

Data relevant to an evaluation of cancer-preventive activity in experimental models are summarized. For each animal species and route of administration, it is stated whether a change in the incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites

are indicated. Negative findings are also summarized. Dose–response relationships and other quantitative data may be given when available.

8.3.3 Mechanism of action

Data relevant to the mechanisms of cancer-preventive activity are summarized.

8.4 Other beneficial effects

When beneficial effects other than cancer prevention have been identified, the relevant data are summarized.

8.5 Carcinogenicity

Normally, the agent will have been reviewed and evaluated within the *IARC Monographs* programme, and that summary is used with the inclusion of more recent data, if appropriate.

8.5.1 Humans

The results of epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized.

8.5.2 Experimental animals

Data relevant to an evaluation of carcinogenic effects in animal models are summarized. For each animal species and route of administration, it is stated whether a change in the incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites are indicated. Negative findings are also summarized. Dose–response relationships and other quantitative data may be mentioned when available.

8.6 Toxic effects

Adverse effects in humans are summarized, together with data on general toxicological effects and cytotoxicity, receptor binding and hormonal and immunological effects. The results of investigations on the reproductive, genetic and related effects are summarized. Toxic effects are summarized for whole animals, cultured mammalian cells and non-mammalian systems. When available, data for humans and for animals are compared.

Structure–activity relationships are mentioned when relevant to toxicity.

9. Recommendations for research

During the evaluation process, it is likely that opportunities for further research will be identified. This is clearly stated, with the understanding that the areas are recommended for future investigation. It is made clear that these research opportunities are identified in general terms on the basis of the data currently available.

10. Evaluation

Evaluations of the strength of the evidence for cancer-preventive activity and carcinogenicity from studies in humans and experimental models are made, using standard terms. These terms may also be applied to other beneficial and adverse effects, when indicated. When appropriate, reference is made to specific organs and populations.

It is recognized that the criteria for these evaluations, described below, cannot encompass all factors that may be relevant to an evaluation of cancer-preventive activity. In considering all the relevant scientific data, the Working Group may assign the agent, or other intervention to a higher or lower category than a strict interpretation of these criteria would indicate.

10.1 Cancer-preventive activity

These categories refer to the strength of the evidence that an agent prevents cancer. The evaluations may change as new information becomes available.

Evaluations are inevitably limited to the cancer sites, conditions and levels of exposure and length of observation covered by the available studies. An evaluation of degree of evidence, whether for a single agent or a mixture, is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of degree of evidence.

Information on mechanisms of action is taken into account when evaluating the strength of evidence in humans and and in experimental animals, as well as in assessing the consistency of results between studies in humans and experimental models.

10.1.1 Cancer-preventive activity in humans

The evidence relevant to prevention in humans is classified into one of the following four categories.

- *Sufficient evidence of cancer-preventive activity*
  The Working Group considers that a causal relationship has been established between use of the agent and the prevention of human cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.
- *Limited evidence of cancer-preventive activity*
  The data suggest a reduced risk for cancer with use of the agent but are limited for making a definitive evaluation either because chance, bias or confounding could not be ruled out with reasonable confidence or because the data are restricted to intermediary biomarkers of uncertain validity in the putative pathway to cancer.
- *Inadequate evidence of cancer-preventive activity*
  The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding a cancer-preventive effect of the agent, or no data on the prevention of cancer in humans are available.
- *Evidence suggesting lack of cancer-preventive activity*
  Several adequate studies of use or exposure are mutually consistent in not showing a preventive effect.

The strength of the evidence for any carcinogenic activity is assessed in parallel. Both cancer-preventive activity and carcinogenicity are identified and, when appropriate, tabulated, by organ site. The evaluation also cites the population subgroups concerned, specifying ages, sex, genetic or environmental predisposing risk factors and the presence of precancerous lesions.

10.1.2 Cancer-preventive activity in experimental animals

Evidence for prevention in experimental animals is classified into one of the following categories.

- *Sufficient evidence of cancer-preventive activity*
  The Working Group considers that a causal relationship has been established between the agent and a decreased incidence and/or multiplicity of neoplasms.
- *Limited evidence of cancer-preventive activity*
  The data suggest a preventive effect but are limited for making a definitive evaluation because, for example, the evidence of prevention is restricted to a single experiment, the agent decreases the incidence and/or multiplicity only of benign neoplasms or lesions of uncertain neoplastic potential or there is conflicting evidence.
- *Inadequate evidence of cancer-preventive activity*
  The studies cannot be interpreted as showing either the presence or absence of a preventive effect because of major qualitative or quantitative limitations (unresolved questions regarding the adequacy of the design, conduct or interpretation of the study), or no data on prevention in experimental animals are available.
- *Evidence suggesting lack of cancer-preventive activity*
  Adequate evidence from conclusive studies in several models shows that, within the limits of the tests used, the agent does not prevent cancer.

10.2 Overall evaluation

Finally, the body of evidence is considered as a whole, and summary statements are made that encompass the effects of the agents in humans with regard to cancer-preventive activity, carcinogenicity and other beneficial and adverse effects, as appropriate.

General Remarks

1. Introduction

The history of non-steroidal anti-inflammatory drugs (NSAIDs) can be traced to ancient Egypt, where an extract of willow bark was used to treat inflammation (Vane *et al.*, 1990; Wright, 1993). The active component of the extract was subsequently identified as the glucoside of salicyl alcohol. Hydrolysis of the carbohydrate moiety produces salicyl alcohol, which can be oxidized to salicylic acid, the actual anti-inflammatory agent. Severe gastric side-effects associated with oral use of sodium salicylate prompted synthesis of its *ortho*-acetyl derivative for use as a possible prodrug, i.e. a pharmacologically inactive precursor that is converted *in vivo* to an active drug, by metabolism or other physiologically active processes (Vane *et al.*, 1990). In fact, acetylsalicylic acid is anti-inflammatory, analgesic and antipyretic but also ulcerogenic. Acetylsalicylic acid was synthesized in 1897 and was mass produced from 1899 by the German company Bayer for the treatment of fever and rheumatism under the commercial name 'Aspirin®[1]'. Subsequently, new, important pharmacological activities have been reported that have been the subject of much basic and clinical investigation. A number of other anti-inflammatory agents have been developed over the past 50 years (Frölich, 1997). These fall into six distinct classes, listed in Table 1.

In the 1970s, Bennett and Del Tacca (1975) and others observed that certain human cancers, including those in the colon and rectum, produced more prostaglandin E_2 than did surrounding normal tissue. They suggested that tumours that overproduce certain prostaglandins promote their own growth and spread. As NSAIDs inhibit prostaglandin synthesis, this theory gave rise to a series of experimental studies in rodents to test whether high doses of these compounds would inhibit or prevent the growth of colon cancers. Most of the NSAIDs tested effectively inhibited colorectal tumours in rats and mice. Whereas these early studies assessed the broad hypothesis that inhibition of prostaglandin synthesis would inhibit the occurrence or progression of neoplasia, subsequent studies have revealed the probable mechanistic complexicity of these processes.

NSAIDs are currently understood to function primarily through a reduction in prostaglandin synthesis by inhibiting the enzyme prostaglandin endoperoxide synthase. This polypeptide enzyme contains both cyclooxygenase and peroxidase activities. It occurs as two isoforms, designated isoforms 1 and 2, which are referred to as cyclooxygenase (COX-1 and COX-2) in the current literature and in this volume. When the term COX is used alone, it denotes a generic property that is conserved in the two isoforms.

Table 1. Some commonly available, conventional non-steroidal anti-inflammatory drugs

Anthranilic acid derivatives	
	Flufenamic acid, mefanamic acid
Indomethacin and related derivatives	
	Indomethacin, sulindac
Oxicams	
	Isoxicam, piroxicam
Phenylalkanoic acid and related derivatives	
	Alclofenac, diclofenac, fenclofenac, flurbiprofen, ibuprofen, ketaprofen, naproxen
Pyrazole derivatives	
	Azapropazone, dipyrone, oxyphenbutazone, phenylbutazone
Salicylates	
	Aspirin, benzorylate, diflunisal, salicylamide, sodium salicylate

[1] Aspirin is a protected trade name of Bayer Company in more than 70 countries

1.1 Observational studies of colorectal cancer

Studies of the effect of NSAIDs on the risk for colorectal cancer initially evolved independently of experimental work on the mechanism of action of these drugs. Kune *et al.* (1988) reported a negative association between the incidence of colorectal cancer and use of aspirin, and reductions of lesser magnitude with the use of other NSAIDs. The potential cancer-preventive activity of aspirin was then evaluated in other epidemiological studies (Gann *et al.*, 1993; Greenberg *et al.*, 1993; Suh *et al.*, 1993; Thun *et al.*, 1993). These are described in the chapter on aspirin.

Studies of cancer occurrence in patients with rheumatoid arthritis in Finland (Isomäki *et al.*, 1978; Laakso *et al.*, 1986) and Sweden (Gridley *et al.*, 1993) were motivated by concern that use of NSAIDs might increase the risk for gastric cancer and that chronic immune stimulation due to rheumatoid arthritis might cause lymphatic or haematopoietic cancers.

1.2 Studies in experimental animals

When the findings in humans were published, there was an extant literature on inhibition of colorectal carcinogenesis by NSAIDs in rodent models (Kudo *et al.*, 1980; Pollard & Luckert, 1980; Narisawa *et al.*, 1981; Pollard & Luckert, 1981a,b; Narisawa *et al.*, 1983; Pollard & Luckert, 1983; Pollard *et al.*, 1983; Narisawa *et al.*, 1984; Pollard & Luckert, 1984; Birkenfeld *et al.*, 1987; Reddy *et al.*, 1987; Moorghen *et al.*, 1988; Reddy *et al.*, 1990; Rao *et al.*, 1991; Reddy *et al.*, 1992). Most of these studies are based on inhibition of tumours induced at particular sites by appropriate chemical carcinogens. Their results suggest that rodent models may be useful for evaluating the chemopreventive activity of NSAIDs and for investigating their mechanisms of action. All of the experimental studies involved single compounds and are thus discussed in the individual chapters.

1.3 Drugs considered

The present volume contains evaluations of the cancer-preventive activity of four specific NSAIDs, together with other relevant findings. The drugs covered are aspirin, indomethacin, sulindac and piroxicam. These NSAIDs were selected because of the amount and nature of the information available on possible cancer-preventive activity. In some studies, NSAIDs or NSAIDs apart from aspirin were addressed as a class. As for many years aspirin was the most commonly used NSAID, studies on NSAIDs in general are covered in that chapter; those on NSAIDs other than aspirin are summarized in the chapter on sulindac. The cancer-preventive activity of NSAIDs as a class was not evaluated.

2. Colorectal cancer

2.1 Descriptive epidemiology

Cancers of the large bowel (colon and rectum) are the third most frequent cancers in the world in people of each sex, after cancers of the lung and stomach in males and after those of the breast and cervix in females. In developed countries, colorectal cancer ranks second; the age-standardized rates are about four times higher than those in developing countries. Thus, about two-thirds of the estimated annual total of 677 000 new cases in 1985 occurred in the developed world, which includes only one-quarter of the world's population (Parkin *et al.*, 1993).

2.2 Biology of colorectal cancer in humans

Malignant tumours are understood to arise from normal cells as a consequence of a multistep process marked by the successive evolution of cell populations with progressively altered growth characteristics. Colorectal cancer is perceived as exemplifying this hypothesis particularly well, because certain intermediate stages in tumour growth are recognized. Aberrant crypt foci are considered to be early preneoplastic lesions that are observed consistently in the colonic mucosa of patients with colorectal cancer (Pretlow *et al.*, 1992). Evidence that several inhibitors of aberrant crypt foci reduce the risk for colorectal tumours in experimental animals suggests that induction of these foci could be used to evaluate new agents for potential preventive properties against colorectal cancer.

Adenomatous colonic polyps are perceived as marking an early stage of cancer development. Patients with an autosomal dominant condition, familial adenomatous polyposis, characterized by the development of hundreds to thousands of polyps, are at greatly increased risk of colorectal

cancer and are offered prophylactic colectomy in early adulthood (Burn *et al.*, 1994; Mills *et al.*, 1997). Mutations in the *APC* gene underlie this phenotype and are demonstrable in the earliest stages of most sporadic cases of colon cancer (Powell *et al.*, 1992; Kinzler & Vogelstein, 1996).

There is good evidence that chronic inflammation predisposes to the development of cancer at various sites. Examples (reviewed by Gordon & Weitzman, 1993) include the associations of urinary bladder cancer with schistosomiasis, stones or in-dwelling catheters left for long periods; oesophageal cancer subsequent to reflux oesophagitis or Barrett's oesophagus; pancreatic cancer subsequent to chronic pancreatitis; gastric cancer following gastritis due to *Helicobacter pylori* and cancer of the gall-bladder subsequent to cholecystitis. The corresponding observation for colorectal cancer is primarily an increased risk in patients suffering from long-standing, extensive inflammatory diseases such as ulcerative colitis. Interestingly, a drug structurally similar to aspirin, aminosalicylate, is used in the treatment of this condition.

The molecular basis of malignant transformation mainly involves the activation of genes that are not usually expressed in normal cells (oncogenes) and loss or mutation of genes associated with the control of cell growth (tumour suppressor genes). Colorectal cancer represents the most comprehensive relationship between genetic changes and successive morphological changes marking the development of malignant tumours. Vogelstein and Kinzler (1993) identified the activation of particular oncogenes, including *ras*, and the loss of particular tumour suppressor genes, including *APC* and *p53*, with various stages in tumour growth: development of hyperplastic lesions, growth of adenomas and development of carcinomas (Fig. 1). Most of the genes that have been associated with the development of colorectal cancer are involved in the control of cell growth and/or the maintenance of genomic stability (Table 2).

ras proto-oncogenes have been the subject of studies relevant to the cancer-preventive activity of some NSAIDs. Thus, recent evidence suggests that activation of *ras* proto-oncogenes, coupled with the loss or inactivation of suppressor genes, induces the malignant phenotype in colonic cells. *ras* proto-oncogenes are functionally related to 21-kDa proteins, ras p21, which are anchored to the cytoplasmic face of the plasma membrane and are believed to function as molecular switches in transmembrane signalling events of cell growth and differentiation (Forrester *et al.*, 1987). By far the most frequent *ras* activation has been observed in codons 12 and 13 of *K-ras*, occurring at a frequency of about 30% in lung adenocarcinomas (Rodenhuis & Slebos, 1992), 40–60% in those in the colon (Burmer *et al.*, 1991) and over 90% in those in the pancreas (Almoguera *et al.*, 1988).

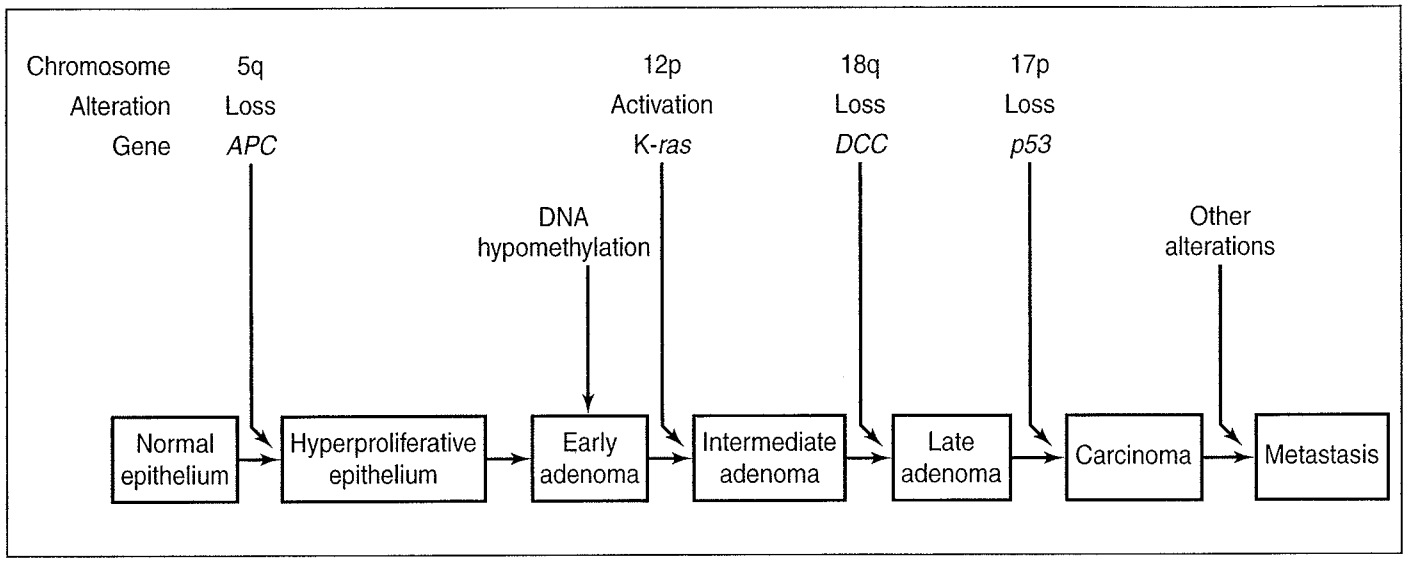
Hereditary non-polyposis colon cancer is an autosomal dominant or recessive condition characterized by the development of multiple cancers at an early age, predominantly in the proximal colon. As adenomas do not occur in large numbers, this syndrome can be distinguished from familial adenomatous polyposis (Jass & Stewart, 1992). Single gene abnormalities leading to hereditary non-polyposis colon cancer may underlie 5–10% of cases of colorectal cancer. The two genes in which causative mutations occur most commonly, *hMLH1* and *hMSH2*, belong to a group responsible for the detection and repair of mismatch mutations during DNA replication (Fishel *et al.*, 1993; Papadopoulos *et al.*, 1994).

Despite the general correlations that have been made between particular genes and the development of carcinomas of the colon, marked heterogeneity is seen between individuals and within tumour cell populations. Thus, some malignant tumours may have only a subset of the genetic changes generally associated with their particular pathological stage. Likewise, although understanding of tumour growth in terms of cellular evolution is widely if not universally accepted, it is not certain that all malignant cancers of the colon develop along this pathway.

2.3 Experimental studies

Knowledge of the cancer-preventive effects of NSAIDs is derived largely from a range of experimental studies. Primary among these are investigations involving administration of these agents to rats and mice treated with chemicals known to cause colon cancer, such as dimethylhydrazine, methylazoxymethanol and azoxymethane. A

Figure 1. Changes that occur during the evolution of a typical colorectal carcinoma in a model of tumour progression in which independent steps are required, leading to the activation of at least one proto-oncogene, coupled with the successive loss of several tumour suppressor genes.



Adapted from Vogelstein & Kinzler (1993)

Table 2. Genes altered in colon cancer

Gene	Chromosome	Tumours with mutations (%)	Class	Action
hMSH2	2	15	Tumour suppressor	DNA repair
K-ras	12	50	Oncogene	Intracellular signalling molecule
Cyclins	Various	4	Oncogene	Regulation of cell cycle
neu/HER2	17	2	Oncogene	Growth factor receptor
myc	8	2	Oncogene	Regulation of gene activity
APC	5	> 70	Tumour suppressor	Regulation of gene activity
DCC	18	> 70	Tumour suppressor	Differentiation signal
p53	17	> 70	Tumour suppressor	Regulation of cell cycle arrest and apoptosis

Adapted from Marx (1993), Vogelstein & Kinzler (1994) and Fazeli *et al.* (1997)

number of specific tumours, such as those of the colon, urinary bladder and kidney, induced in rodents by carcinogens are known to mimic the histopathological development of the corresponding human cancers. These 'model' systems are therefore used in various aspects of cancer research. The relevance of animal models for human colorectal cancer is indicated by the occurrence in both of the adenoma–carcinoma sequence and by the similarity of histopathological appearance; furthermore, mutations in the *ras* oncogene at codons 12 and 13 are found in colorectal tumours from both humans and experimental animals. Many of the relevant studies are predicated on administration of the maximum tolerated dose (MTD) of an agent, which is defined as the highest dose that causes no more than a 10% weight decrement in comparison with appropriate controls and does not induce mortality or any clinical signs of toxicity that would be predicted to shorten the natural lifespan of the animal.

Experimental models of colorectal cancer have also provided information on the early stages of tumour growth. Thus, aberrant crypt foci have been recognized as early indicators of tumour development in experimental animals (McLellan

et al., 1991; Rao *et al.*, 1993; Wargovich *et al.*, 1995), and several models of familial adenomatous polyposis exist in which a mouse strain carries different mutations in the *Apc* gene. The first of these was the Min mouse (Moser *et al.*, 1990; Su *et al.*, 1992; Moser *et al.*, 1993). The Min/+ mouse carries a fully penetrant dominant mutation at codon 850 of the murine *Apc* gene and develops adenomas throughout the intestinal tract, mostly in the small intestine, without treatment by a carcinogen. The phenotypic expression of an *Apc* mutation in the Min/+ mouse is thus different from that of humans with familial adenomatous polyposis, in whom adenomas are found exclusively in the colon and duodenum. Nevertheless, this model has been used to study the chemopreventive properties of several NSAIDs. A molecular basis for the association between inflammation and the growth of colonic polyps has also been demonstrated. Studies of mice carrying an *Apc* mutation which were cross-bred with animals with a disrupted *Cox-2* gene demonstrated that induction of *Cox-2* is an early, rate-limiting step in adenoma formation (Oshima *et al.*, 1996). Adenomas taken from Min/+ mice have a high level of *Cox-2* expression (Williams *et al.*, 1996).

Cell culture systems have also been used to study the possible chemopreventive activity of NSAIDs. Much of the work was carried out with cultured intestinal epithelial cells and colon cancer cell lines. Such studies have shown both prostaglandin-dependent and -independent effects of parent compounds and, in some instances, their metabolites. When interpreting the results of these studies, it is vital to consider the experimental context in which they were generated, particularly with regard to the concentration of the agent and the specific model used. For example, many of the non-prostaglandin effects of NSAIDs shown in these systems may occur only at concentrations of the agent that are unachievable under physiological conditions *in vivo*. In addition, human colorectal tumour cells represent a late stage in the adenoma–carcinoma sequence; therefore, any observed effects may not represent the true mechanism (Kargman *et al.*, 1995).

NSAIDs cause apoptosis when applied to *v-src*-transformed chicken embryo fibroblasts (Lu *et al.*, 1995). This study was the first to indicate that cells that overexpress *Cox-2* may be somewhat resistant to programmed cell death and that this can be reversed by addition of an NSAID.

Few nontransformed intestinal epithelial cell lines are available for in-vitro studies. Cell lines have successfully been derived from the rat small intestine and placed in continuous culture, and several groups have evaluated the mechanisms for growth control of the rat intestinal epithelial-1 (RIE-1) cell line (DuBois *et al.*, 1995). Sulindac sulfide can inhibit mitogenesis of these cells in culture. Additionally, these cells express the inducible Cox-2 enzyme after treatment with cytokines and growth factors (DuBois *et al.*, 1994). The increased expression of Cox-2 alters their apoptotic phenotype and makes them resistant to programmed cell death (Tsujii & DuBois, 1995).

3. Pharmacological action of non-steroidal anti-inflammatory drugs

3.1 Synthesis and action of prostaglandins

Central to the understanding of the pharmacology of NSAIDs is the effect of these agents on prostaglandin synthesis (see the box for explanation of relevant terms).

COX catalyses the biosynthesis of prostaglandins and thromboxanes, which are bioactive lipids that play a role in a broad range of physiological and pathophysiological processes (Hamberg & Samuelsson, 1967; Needleman *et al.*, 1986). COX has two enzymatic activities, a cyclooxygenase activity that oxygenates arachidonic acid to a hydroperoxy endoperoxide, prostaglandin G_2, and a peroxidase that reduces prostaglandin G_2 to the hydroxy endoperoxide, prostaglandin H_2 (Ohki *et al.*, 1979).

NSAIDs act by binding tightly at the cyclooxygenase active site, preventing combination of the enzyme with arachidonic acid (Picot *et al.*, 1994; Loll *et al.*, 1995, 1996). Aspirin is unique among the NSAIDs in that it covalently modifies the protein by transferring its acetyl group to a serine hydroxyl group at the cyclooxygenase active site (Van Der Ouderaa *et al.*, 1980). The acetylation is irreversible, so that aspirin treatment permanently disables COX until it is regenerated. All of the other NSAIDs bind reversibly to the protein. All of the commercially available NSAIDs, including aspirin, inhibit both COX-1 and COX-2 although the extent of inhibition differs (Meade *et al.*, 1993;

Terms used in describing prostaglandin synthesis

COX-1	Cyclooxygenase-1, a physiologically expressed enzyme that can convert arachidonic acid into prostaglandins and thromboxanes. Also called prostaglandin H synthase-1
COX-2	Cyclooxygenase-2, an inducible enzyme that is often up-regulated at sites of inflammation. Also called prostaglandin H synthase-2
Prostaglandin	A class of physiologically produced substances with effects such as vasodilatation (e.g. prostaglandin E_2) and vasoconstriction (prostaglandin F_2), which are believed to play an important role in the process of inflammation
Prostanoids	Any of several complex fatty acids 20 carbons in length, derived from arachidonic acid and containing an internal 5- or 6-carbon ring. Include prostaenoic acid, prostaglandins and thromboxanes
Eicosanoids	Physiologically active substances derived from arachidonic acid, e.g. prostaglandins, leukotrienes and thromboxane
Autocoid	Chemical produced by one type of cell which affects the functioning of different cells in the same region
Arachidonic acid	An unsaturated fatty acid essential in nutrition; the biological precursor of prostaglandins, thromboxanes and leukotrienes
Thromboxane	A series of compounds formed directly from prostaglandins; the name is derived from their physiological effect on platelet aggregation
Lipoxygenase	An enzyme that can convert arachidonic acid and other unsaturated fatty acids into leukotrienes
Leukotriene	Lipoxygenase enzyme product with postulated role in inflammation and allergy; differs structurally from the related prostanoids by the absence of a central ring

Laneuville *et al.*, 1994; O'Neill *et al.*, 1994; Gierse *et al.*, 1995).

The emphasis in this volume is mainly on prostaglandins, since inhibition of their synthesis is known to be a common mechanisms of action of NSAIDs (Vane, 1971); however, other metabolites of arachidonic acid, such as the leukotrienes and lipoxins, may also affect tumorigenesis (Marnett, 1992). Furthermore, other eicosanoids such as eicosopentanoic acid, may also be metabolized by cyclooxygenases and peroxidases to prostaglandins (Fig. 2).

COX-1 is expressed constitutively in a number of cell types and tissues, including gastric mucosa (Williams & DuBois, 1996). In contrast, *COX-2* belongs to a class of genes referred to as 'immediate early' or 'early growth response' genes, which are expressed rapidly and transiently after stimulation of cultured cells by growth factors, cytokines and tumour promoters (Nathans *et al.*, 1988; Herschman, 1991); *COX-2* expression is thus increased in inflammatory cells and at sites of inflammation (Masferrer *et al.*, 1994). It is also increased in malignant colorectal epithelial cells, fibroblasts and tumour vascular endothelium (Sano *et al.*, 1995).

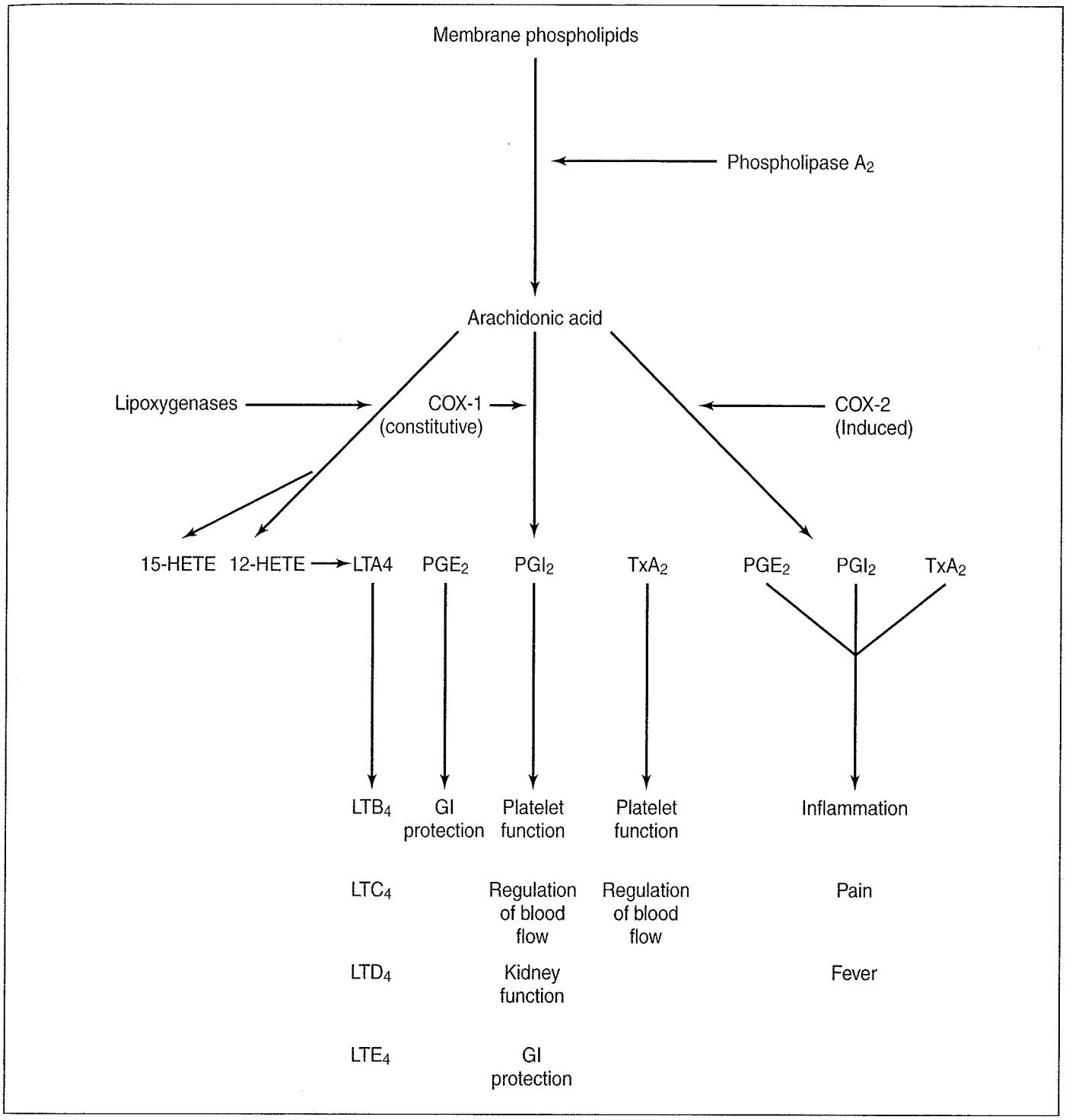
The identification of two different forms of COX and their differential tissue distribution raised the possibility that selective COX inhibitors could be developed that would modulate the catalytic activity of only one of the forms. For example, one might expect COX-2-selective inhibitors to be anti-inflammatory and analgesic but to lack the gastrointestinal complications that are responsible for the dose-limiting toxicity of the currently available NSAIDs (DeWitt *et al.*, 1993).

3.2 Prostaglandins and human tumours

Numerous reports published over the past two decades provide overwhelming evidence of a significant association between prostaglandins and carcinogenesis (Jaffe, 1974; Lupulescu, 1978a,b; Karmali, 1980; Honn *et al.*, 1981). Increased levels of prostaglandins, most notably E_2, have been detected in many malignant tumours. Prostaglandins stimulate tumour growth and dramatically increase DNA and RNA synthesis in cancer cells (Lupulescu, 1975, 1977, 1978b). They presumably act as co-carcinogens by enhancing the rate of tumour progression (Lupulescu, 1978a). The finding that prostaglandins bind to nuclear chromatin and alter DNA synthesis further supports the hypothesis that they are tumour promoters (Lupulescu, 1980).

Increased levels of prostaglandins E_2, $F_{2\alpha}$ and all prostaglandins have been documented in medullary carcinoma of the thyroid (Williams *et al.*, 1968), ganglioneuroma, neuroblastoma, phaeochromocytoma, carcinoids (Sandler *et al.*,

Figure 2. Biosynthesis of eicosanoids



COX, cyclooxygenase; PG, prostaglandin; LT, leukotriene; Tx, thromboxane; HETE, hydroeicosatetraenoic acid; GI, gastrointestinal

1968), Kaposi's sarcoma (Bhana *et al.*, 1971), renal-cell carcinoma (Cummings & Robertson, 1977), lung cancer (Sandler *et al.*, 1968; Hubbard *et al.*, 1988), oesophageal carcinomas (Botha *et al.*, 1986), squamous-cell carcinomas of the head and neck (El Attar & Lin, 1987), breast cancers (Bennett *et al.*, 1977a, 1979) and colorectal cancers (Jaffe, 1974; Bennett & Del Tacca, 1975; Bennett *et al.*, 1977b; Lange *et al.*, 1985; Rigas *et al.*, 1993). In later studies, arachidonate, the substrate from which prostanoids are derived (Bennett *et al.*, 1987) and other eicosanoid metabolites were measured in various tumours (Dreyling *et al.*, 1986; Shimakura &

Boland, 1992; Marnett, 1992; Ghosh & Myers, 1996).

In some tumours, the concentrations of prostaglandins measured in homogenized samples of tissue or in venous blood draining from the tumour correlate with its size and/or invasiveness. Narisawa *et al.* (1990) observed that the level of prostaglandin E_2 in blood from human colorectal cancers was higher when the cancers were large or locally invasive. Similarly, Klapan *et al.* (1992) related plasma prostaglandin E_2 concentrations to the invasiveness of head-and-neck cancers. Several studies have shown that breast nodules contain more total prostanoids when they are malignant, and even more when they are metastatic or associated with shorter survival (Bennett *et al.*, 1977a, 1979, Rolland *et al.*, 1980).

The physiology of eicosanoids is complicated, however, in that the same autocoid may have opposite effects in different organs. For example, prostaglandin E_2 inhibits tumour growth in gastric KATO III and AGS carcinoma cell lines (Nakamura *et al.*, 1991; Shimakura & Boland, 1992), yet appears to promote tumorigenesis at other sites. Such organ specificity may be analogous to the protective function of prostaglandin E_2 in the human stomach (Miller, 1983), despite its contribution to inflammation elsewhere.

It is also uncertain whether tumour epithelial cells or inflammatory cells are important producers of prostaglandins. Only one of three human colorectal cancer cell lines (adenocarcinomas and carcinomas) produced detectable eicosanoid levels in a survey by Hubbard *et al.* (1988). Tissue-fixed macrophages produce prostaglandin E_2 in colorectal cancers (Maxwell *et al.*, 1990), and nonmalignant fibrous tissue was associated with increased prostaglandin synthesis in colorectal mucosa (Bennett *et al.*, 1977b).

The conclusion that the increased activity of COX-2 and its prostaglandin products plays a direct role in tumorigenesis is suggested by a number of observations.

It has been demonstrated that *COX-2* expression is greater in human colorectal adenocarcinomas than in adjacent normal colonic mucosa (Eberhart *et al.*, 1994; Gustafson-Svärd *et al.*, 1996; Kutchera *et al.*, 1996), and it increases progressively from 40–50% in colorectal adenomas to 85–90% in carcinomas (Eberhart *et al.*, 1994). These findings have been confirmed by other investigators, who have shown elevated levels of COX-2 protein in colorectal tumours by western blotting (Kargman *et al.*, 1995) and immunohistochemical staining (Sano *et al.*, 1995). COX-2 is found in some (Caco-2, LoVo) but not all (LS123, SW480) cell lines of human colorectal cancers (Hecht *et al.*, 1995). Experiments conducted on transfected colon tumour cells indicate that overexpression of *COX-2* may be due partially to abnormal constitutive transcription of the *COX-2* promoter (Kutchera *et al.*, 1996). There are markedly elevated levels of *Cox-2* messenger RNA and protein in colonic tumours that develop in rodents after treatment with a carcinogen (DuBois *et al.*, 1996a) and in adenomas taken from Min mice (Williams *et al.*, 1996). These observations of elevated *Cox-2* expression in three different models of colorectal carcinogenesis have led to the hypothesis that *COX-2* expression is causally related to colorectal tumorigenesis. A recent study demonstrated a 40–49% reduction in aberrant crypt formation in carcinogen-treated rats that were given a selective Cox-2 inhibitor (Reddy *et al.*, 1996). Another report provides genetic evidence that directly links *Cox-2* expression to intestinal tumorigenesis (Oshima *et al.*, 1996; Prescott & White, 1996). In this study, mice lacking the *Apc* gene were generated, which developed hundreds of tumours per intestine. When these mice were bred with mice lacking *Cox-2*, there was an 80–90% reduction in tumour multiplicity in the homozygous null offspring. These results point to two important findings: (i) Cox-2 may act as a tumour promoter in the intestine, and (ii) increased levels of *Cox-2* expression may result directly from disruption of the *Apc* gene (Prescott & White, 1996). These results clearly demonstrate that COX-2 is a feasible target for future strategies for the prevention and treatment of colorectal cancer.

Although there is strong evidence that inhibition of COX (especially, COX-2) contributes to the ability of NSAIDs to inhibit the development of colorectal cancer, the mechanisms by which *COX* expression contributes to tumorigenesis are unclear. Prostaglandins and thromboxane, the products of arachidonic acid oxygenation via the cyclooxygenase pathway, have diverse biological effects, including stimulation of cell proliferation,

suppression of the immune response and alteration of haemodynamic properties (Marnett, 1992). Overexpression of *Cox-2* in rat intestinal epithelial cells induces a delay in G_1 and renders them resistant to the induction of apoptosis by sodium butyrate (Tsujii & DuBois, 1995; DuBois *et al.*, 1996b). Each prostaglandin and thromboxane has a specific *trans*-membrane, G-protein-linked receptor coupled to an intracellular signalling pathway. Thus, there are multiple mechanisms by which the products formed from COX could enhance the growth of transformed colonic epithelial cells.

3.3 Alternative mechanisms

3.3.1 Mechanisms independent of prostaglandins

Several lines of evidence indicate that the mechanism of action of NSAIDs is not mediated by prostaglandins. Their ability to inhibit growth and block cell differentiation has been shown in several tumour cell lines (Santoro *et al.*, 1976; Tutton & Barkla, 1980; Karmali, 1983). Furthermore, prostaglandin-induced inhibition of DNA synthesis has been reported (Craven *et al.*, 1983). In another investigation, the concentrations of NSAIDs that inhibited the growth of human fibroblasts and rat hepatoma cell lines were poorly correlated with the levels reported in other studies (DeMello *et al.*, 1980). Other reports suggest that NSAIDs induce apoptosis in colon tumour cells, including some lines that do not express COX or make prostaglandin (Elder *et al.*, 1996; Hanif *et al.*, 1996). Thus, NSAIDs may exert their chemopreventive effects on colon tumour cells by a combination of prostaglandin-dependent and -independent mechanisms.

Other possible actions include alternative facilitation of the formation of hydroxy eicosatetranoic acid (see Fig. 2), reduction of phosphodiesterase and protein kinase activity and modulation of immune and angiogenic responses (Benamouzig *et al.*, 1997). The high levels of salicylic acid in plants have been shown to be related to the induction of apoptosis in response to infection (Hunt *et al.*, 1996). At the site of invasion of a pathogen in plants, programmed cell death is the primary response in order to localize the disease. In addition, plants possess an inducible resistance mechanism, which is dependent upon accumulation of salicylic acid (Delaney *et al.*, 1994). This system enhances apoptosis at other sites in response to the same infectious agent. A study in the Netherlands demonstrated negligible quantities of salicylic acid in the current western diet, presumably as a result of agricultural control of plant pathogens (Janssen *et al.*, 1996).

The accumulation of genetic mutations in specific tissues appears to play an important role in malignant transformation. One well-known directly acting mutagen and lipid peroxidation product, malondialdehyde, can be generated by the cyclooxygenase pathway via enzymatic and non-enzymatic degradation of prostaglandin H_2 and also by lipid peroxidation of polyunsaturated fatty acids (Hamberg & Samuelsson, 1967; Mukai & Goldstein, 1976; Diczfalusy *et al.*, 1977). The mutagenicity of malondialdehyde has been demonstrated in several bacterial and mammalian systems (Mukai & Goldstein, 1976; Basu & Marnett, 1983), and its carcinogenic potential has been documented in rats (Spalding, 1988). Moreover, in human colorectal tumours, elevated levels of malondialdehyde were significantly correlated with prostaglandin E_2 concentrations (Hendricks *et al.*, 1994). Thus, prevention of malondialdehyde-induced mutagenesis may be yet another indirect mechanism relevant to the cancer-preventive action of NSAIDs.

3.3.2 Effects on non-cancerous tissues

Any link between up-regulated COX expression in cancerous tissue and the initial development of that cancer is unclear. However, COX-2 is also up-regulated in inflammatory tissues, and there is good evidence that inflammation due to, for example, *Helicobacter* gastritis and ulcerative colitis, is associated with cancer development. In the latter case, any reduction in the risk for colorectal cancer by aminosalicylate may thus be related to suppression of inflammatory activity rather than to an intrinsic effect on cell behaviour (Mulder *et al.*, 1996). It remains to be determined whether pharmacological down-regulation of induced COX-2 in inflamed tissues would interfere with any propensity to develop cancer. In this context, it should be noted that nitric oxide, another inflammatory mediator, may be involved in multistage carcinogenesis (Ohshima & Bartsch, 1994), and nitric oxide appears to activate COX enzymes (Salvemini *et al.*, 1993).

3.3.3 Cytochrome P450

A further possible mechanism is through inhibition of cytochrome P450 monooxygenase activity. Activation of the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by some NSAIDs has been claimed to occur through inhibition of this enzyme (Bilodeau *et al.*, 1995).

Co-oxidation of xenobiotics through a COX-mediated mechanism has been the subject of several recent reviews (Eling *et al.*, 1990; Marnett, 1990; Smith *et al.*, 1991). While the possibility was raised that this pathway represents an alternative to the cytochrome P450 metabolizing enzymes, it is now generally accepted that it does not play a significant role in systemic drug metabolism; however, data linking urinary bladder cancer to the oxidation of aromatic amines in this tissue (Rice *et al.*, 1985) suggest that COX plays a role in producing tissue-specific carcinogens (Marnett, 1990).

A wide variety of potential environmental carcinogens, including polycyclic aromatic hydrocarbons, hydrazine, aromatic amines and phenols, are oxidized through the peroxidase activity of COX (Boyd & Eling, 1987; Reed, 1988; Schlosser *et al.*, 1989; Eling *et al.*, 1990; Smith *et al.*, 1991). Co-production of active oxygen species such as superoxide anions, hydrogen peroxide and hydroxyl radicals which are known to damage cellular DNA, may contribute to tumour initiation and promotion (Ames *et al.*, 1993; Gordon & Weitzman, 1993). Indeed, the peroxidase-generating activity of COX has been implicated in the toxicity of several xenobiotics, including benzene (Gaido & Weirda, 1987; Pirozzi *et al.*, 1989). COX catalyses conversion of the benzene metabolite hydroquinone into reactive oxygen products that accumulate in bone marrow and damage DNA (Lewis *et al.*, 1988).

4. Adverse effects

Many studies have shown that NSAIDs at doses used for the treatment of arthritis increase the risk for ulcer complications by two- to five-fold (Gabriel *et al.*, 1991; Bollini *et al.*, 1992; Henry *et al.*, 1996). It has been estimated in several populations that 15–35% of all ulcer complications are due to these drugs (Somerville *et al.*, 1986; Griffin *et al.*, 1988, 1991; Henry *et al.*, 1991; Laporte *et al.*, 1991; Gutthann *et al.*, 1994; Langman *et al.*, 1994). In the United States alone, there are an estimated 24 000 hospitalizations and 2600 deaths annually among patients with rheumatoid arthritis (Fries *et al.*, 1991a).

The rates of hospital deaths associated with peptic ulcer disease are 2–10% (Laporte *et al.*, 1991; Savage *et al.*, 1993; Garcia Rodriquez & Jick, 1994; Gutthann *et al.*, 1994), the higher estimates being those for older populations. The rate of fatal complications in elderly NSAID users is close to 1 per 1000 person-years of NSAID use; it is higher for those with additional risk factors such as a prior history of ulcer disease.

Variations in both the dose and duration of use of NSAIDs and in host factors are important in determining the rates of disease for individuals. Initial use and use of higher doses have been associated with higher risks for adverse effects (Griffin *et al.*, 1991). In a large meta-analysis (Henry *et al.*, 1996), most of the 12 NSAIDs evaluated were statistically indistinguishable with regard to the risk for ulcer complications; however, some were consistently associated with higher rates of such complications in individual studies. Henry *et al.* attempted to find a ranking order that best summarized the sequence of risks observed. In this analysis, with 1 being the most and 12 the least toxic, aspirin ranked 5.

The combination of NSAIDs and oral corticosteroids increased the risk for ulcer complications by 13–15 times that of non-users of either drug, such that older persons using this combination of drug have a hospitalization rate for ulcers of 5–6% per year (Piper *et al.*, 1991; Gutthann *et al.*, 1994).

Fries *et al.* (1991b) based a toxicity index for NSAIDs on the frequency and severity of a variety of adverse events among 2747 patients with rheumatoid arthritis receiving 11 different NSAIDs. The signs and symptoms (including rashes, oedema, central nervous system symptoms and gastrointestinal symptoms) were weighted to develop a score for each NSAID. This score was also adjusted for a large number of potentially confounding variables, including age, sex, other drug use and other measures of co-morbidity and disability. Aspirin (mean dose, 2415 mg) had the lowest adjusted toxicity score; sulindac (379 mg) and piroxicam (19 mg) had intermediate scores, with ranks of 5 and 6, respectively; and

indomethacin (100 mg) had the highest score. The latter was heavily influenced by central nervous system symptoms, whereas the ranking of the other NSAIDs was influenced more strongly by gastrointestinal symptoms and complications.

NSAID treatment has been associated with stricture, acute bleeding, perforation and chronic inflammatory lesions in both the small and large bowel (Langman *et al.*, 1985; Ravi *et al.*, 1986; Bjarnason *et al.*, 1987a,b; Kaufmann & Taubin, 1987; Rampton, 1987; Banerjee, 1989). NSAIDs also cause dose-dependent increases in the frequency and severity of dyspepsia, haemorrhage and perforation. Death may ensue (Griffin *et al.*, 1988; Guess *et al.*, 1988; Gabriel *et al.*, 1991; Griffin *et al.*, 1991; Garcia-Rodriquez *et al.*, 1992; Smalley *et al.*, 1995). Symptoms in the upper gastrointestinal tract are common and are a frequent reason both for withdrawal of NSAIDs and for concomitant treatment with H_2 receptor antagonists, antacids, sucralfate or misoprostol (Hogan *et al.*, 1994; Smalley *et al.*, 1996).

Gastrointestinal lesions may result from the drug's direct action on the mucosa through oxygen radical-mediated lipid peroxidation and the subsequent accumulation of lipoxygenase and leukotriene products resulting from the shift of the arachidonic acid cascade into the 5-lipoxygenase pathway (Lippmann, 1974; Gaffney & Williamson, 1979; Cronen *et al.*, 1982; Ligumsky *et al.*, 1983; Flower *et al.*, 1985; Lippton *et al.*, 1987; Meyers *et al.*, 1991; Kapui *et al.*, 1993; Yoshikawa *et al.*, 1993; Zahavi *et al.*, 1995; Elliot *et al.*, 1996; Rioux & Wallace, 1996; Takeuchi *et al.*, 1996).

Although the gastrointestinal adverse effects of NSAIDs form by far the commonest and most important variety, there is a range of others. They include toxic hepatitis, blood dyscrasias, skin disorders, interstitial nephritis and cystitis. In general, these are rare and in some cases appear to be largely restricted to particular drugs.

4.1 Ulceration

Host factors that increase the rate of serious ulcer disease include older age (Garcia Rodriguez & Jick, 1994; Gutthann *et al.*, 1994), history of prior ulcer, gastrointestinal haemorrhage, dyspepsia and previous intolerance to NSAIDs (Fries *et al.*, 1991; Garcia Rodriguez & Jick, 1994; Gutthann *et al.*, 1994; Silverstein *et al.*, 1995), use of corticosteroids (Fries *et al.*, 1991a; Piper *et al.*, 1991; Garcia Rodriguez & Jick, 1994; Gutthann *et al.*, 1994), use of anticoagulants (Shorr *et al.*, 1993; Garcia Rodriguez *et al.*, 1994) and various measures of poorer health (Fries *et al.*, 1991a; Griffin *et al.*, 1991; Silverstein *et al.*, 1995).

4.1.1 Age

Hospitalizations for ulcer disease have been reported to be fewer than 1 per 1000 annually in most populations under the age of 60 years (Schoon *et al.*, 1989; Steering Committee of the Physicians' Health Study Research Group, 1989; Henry & Robertson, 1993; Johnson *et al.*, 1994a; Garcia Rodriguez & Jick, 1994). The rates increase with age, however, so people aged 65 years and older have incidences of 2–6 per 1000 (Laporte *et al.*, 1991; Garcia Rodriguez *et al.*, 1992; Graves & Kozak, 1992; Henry & Robertson, 1993; Shorr *et al.*, 1993; Smalley *et al.*, 1995). The higher absolute rate of ulcer complications associated with age has been observed consistently (Fries *et al.*, 1991a; Garcia Rodriguez *et al.*, 1992; Garcia Rodriguez & Jick, 1994; Gutthann *et al.*, 1994; Lanza *et al.*, 1995; Silverstein *et al.*, 1995).

For persons 65 years and older enrolled in the Tennessee Medicaid programme, the annual number of hospitalizations for ulcer was approximately 4 per 1000 person-years among non-users and 17 per 1000 among NSAID users (Smalley *et al.*, 1995). Because the rate of ulcer disease is much higher in older persons, multiplying this relatively high rate by a factor of 4 has a much greater impact than for populations with a low baseline rate. For patients with rheumatoid arthritis treated with NSAIDs, the incidence of hospitalization or death from acute gastrointestinal events increased from 3 to 19 to 42 per 1000 person–years of use among patients aged < 63, 63–75 and > 75 years, respectively (Fries *et al.*, 1991a).

4.1.2 History of ulcer disease

In most studies, NSAID use by patients with a past history of ulceration, haemorrhage or dyspepsia was associated with a lower relative risk than for patients without such a history (Henry *et al.*, 1996). The risk for ulcer complication in patients who both used NSAIDs and had a history of ulcer

disease was 14–17 times that of patients who had neither of these factors (Garcia Rodriguez & Jick, 1994; Gutthann *et al.*, 1994).These relative risks are consistent with annual rates of hospitalization or death from gastrointestinal disease of 4–8% in a cohort of arthritis patients with both these factors (Fries *et al.*, 1991b).

4.1.3 Helicobacter pylori

Helicobacter pylori and NSAIDs are the major independent causes of both gastric and duodenal ulcers (Borody *et al.*, 1991; Nensey *et al.*, 1991; Borody *et al.*, 1992). Although *H. pylori* infection may not increase the risk for NSAID-associated ulcers (Graham *et al.*, 1991; Loeb *et al.*, 1992), *H. pylori* infection identifies persons with a past history and a higher risk for ulcer disease. Persons with both of these factors have a much higher rate of ulcer disease than those with neither of these factors (Martin *et al.*, 1989). It remains plausible that eradication of *H. pylori* in people with proven ulcer disease (current or past) would reduce the risk for NSAID-induced gastroduodenal ulceration and bleeding.

4.1.4 Corticosteroids

Oral corticosteroids, even at relatively low doses have been reported to double the rate of serious ulcer disease (Fries *et al.*, 1991a; Piper *et al.*, 1991; Garcia Rodriguez & Jick, 1994; Gutthann *et al.*, 1994). The combination of NSAIDs and oral corticosteroids increases the risk for ulcer complication by 13–15 times that of non-users of either drug, such that older persons using this combination of drugs have a hospitalization rate for ulcer of 5–6% per year (Piper *et al.*, 1991; Gutthann *et al.*, 1994).

4.1.5 Anticoagulants

Anticoagulants have no known or postulated ulcerogenic effect, yet they have a profound effect on bleeding. In the outpatient setting, these drugs increase the risk for upper gastrointestinal bleeding by three- to sixfold (Garcia Rodriguez & Jick, 1994; Shorr *et al.*, 1993). The combination of NSAIDs and anticoagulants greatly increases the rate of such complications, such that older person using this combination have a rate of hospitalization for upper gastrointestinal haemorrhage of about 3% per year (Shorr *et al.*, 1993).

4.2 Asthma

About 10% of adults with asthma develop acute, idiosyncratic bronchoconstriction after ingesting aspirin and other NSAIDs (Fischer *et al.*, 1994; Staton *et al.*, 1996). Often called 'aspirin-sensitive' asthma, this condition can be precipitated by currently available NSAIDs (Szczeklik, 1994). The symptoms begin within 15 min to 4 h (usually 1 h) after NSAID ingestion, and may include rhinorrhoea, conjunctival irritation, scarlet flushing of the head and neck and severe, even life-threatening asthma. The respiratory symptoms may continue beyond discontinuation of NSAIDs.

4.3 Drug interactions

Drug interactions may result in inhibition of drug metabolism or displacement of protein binding. Battellino *et al.* (1990) used the elimination rate of antipyrine to estimate the ability of a subject to biotransform drugs metabolized mainly through the oxidative reactions of the cytochrome P450 system. They found that antipyrine metabolism was impaired by concurrent administration of piroxicam. Therefore, drugs may accumulate and become toxic when piroxicam is administered simultaneously with steroid hormones or other compounds metabolized by the mixed-function oxidase system. Increased cytochrome P450 content and aryl hydrocarbon hydroxylase activity have been observed under such conditions (Mostafa *et al.*, 1990). Long-term administration of piroxicam may thus affect the intensity and duration of action of environmental carcinogens that are metabolized by the cytochrome P450-dependent monooxygenase system.

NSAIDs interact with many drugs, as reviewed by Verbeeck (1990; see also Table 3). A number of drugs, including warfarin, diazepam and ibuprofen, may competitively displace piroxicam from its serum albumin binding (Matsuyama *et al.* 1987; Brée *et al.*, 1990). The activities of lithium, methotrexate and, to a lesser extent cyclosporin may be affected by concomitant administration of an NSAID. Interaction with oral anticoagulants, oral antihyperglycaemic agents and the anticonvulsants phenytoin and valproic acid (sodium valproate) could lead to increased NSAID levels in blood. This is potentially dangerous, since high systemic concentrations of NSAIDs may reach the stomach and kidney and mediate toxicity.

Table 3. Therapeutic agents that can interact with non-steroidal anti-inflammatory drugs

Adsorbent antidiarrhoeal drugs
Antacids
Antihypertensive drugs
Cholestyramine
Cimetidine
Cisapride
Cyclosporin
Digoxin
Domperidone
Famotidine
Lithium
Methotrexate
Metoclopramide
Mucoprotective agents
Phenytoin
Ranitidine
Valproic acid
Warfarin

From Said & Foda (1989); Verbeeck (1990); Milligan *et al.* (1993); Brouwers & de Smet (1994); Koytchev *et al.* (1994); Combe *et al.* (1995); Mené *et al.* (1995)

Interactions with digoxin are most likely to occur in the elderly, newborns and patients with renal impairment. Piroxicam can interact with anticoagulant coumarin drugs, can influence thrombocyte aggregation and can enhance bleeding, especially in patients with risk factors such as advanced age, chronic or acute peptic ulcer and use of corticosteroids.

NSAIDs have been shown to antagonize the action of most antihypertensive agents, so that their doses must be increased; however, concomitant administration of NSAIDs often prevents optimal control of blood pressure, particularly among black and elderly patients (Mené *et al.*, 1995). Piroxicam and indomethacin had the most marked effects and sulindac and aspirin the least (Johnson *et al.*, 1994b). The mechanism of the interaction is unknown.

4.4 Taking account of toxic effects

The strength of the evidence for the value of individual NSAIDs in preventing colorectal cancer will necessarily vary according to the intensity and breadth of searches for the beneficial effects obtainable with individual agents, of comparisons between agents and of examinations and comparisons of toxic effects. Although NSAIDs have many common structural features and anti-inflammatory, analgesic and antipyretic properties, they can differ notably with regard to the doses normally employed, their clinical pharmacological and pharmacokinetic characteristics and their toxicity.

Although the choice of a conventional NSAID should be among those for which there is the best evidence of efficacy and lack of toxicity, such information is not yet available. Most of the epidemiological studies addressed NSAIDs as a class or separated only aspirin and non-aspirin NSAIDs, and most of the clinical studies, mainly in patients with familial adenomatous polyposis, investigated sulindac. Although some studies indicate large differences between NSAIDs in their toxicity to the upper gastrointestinal tract, these findings may reflect anti-inflammatory potency and dosage rather than any intrinsic difference in toxicity. Investigations in experimental animals are of limited value for making a choice, as opposed to demonstrating effects and elucidating mechanisms, as few cross-comparisons can be made and direct application of the findings to humans cannot be assumed.

4.5 Mitigating side-effects

Alternative strategies for minimizing toxicity include the co-administration of agents such as hyposecretory drugs, which can reduce the risk for peptic ulceration, and use of more selective NSAIDs which inhibit COX-2.

A range of drugs, including histamine H_2 antagonists, proton pump inhibitors and synthetic prostaglandins, have been shown to reduce, to varying degrees, the risk for developing an ulcer during concurrent use of NSAIDs. Each has potential benefits and risks (Wallace, 1997). Histamine H_2 antagonists are more effective in reducing the frequency of duodenal than gastric ulceration (Ehsanullah *et al.*, 1988). Potency and dosage may partly explain this divergence, since famotidine at high doses appears to be more effective than ranitidine in diminishing the risk for gastric ulcer (Taha *et al.*, 1996). Concern about the potential hazards of long-term use of H_2 antag-

onists now appears to be unfounded (Johnson *et al.*, 1996).

Proton pump inhibitors can prevent both gastric and duodenal ulceration (Scheiman *et al.*, 1994), but good evidence of long-term safety is lacking. The intense inhibition of acid output associated with use of drugs like omeprazole has been associated with a clear elevation of serum gastrin levels, and correlations have been noted between serum gastrin concentration and propensity for colon cancer (Watson *et al.*, 1995). Synthetic prostaglandins such as the prostaglandin E_1 analogue, misoprostil, have licensed indications in the prevention of damage to the upper gastrointestinal tract caused by NSAIDs. Instituting treatment with synthetic prostaglandins while at the same time evaluating whether suppression of their production by NSAIDs is useful would, however, be aberrant.

Newer NSAIDs which, to a greater or lesser degree, inhibit only COX-2 activity are particularly interesting because they could reduce upper gastrointestinal damage, which is probably dependent on suppression of COX-1. These newer drugs cannot, however, be assumed to be free of toxicity. Chemopreventive strategies may imply years of treatment of individuals many of whom may not have developed the disease under scrutiny. Detailed consideration of overall risk–benefit relationships will thus be important.

5. Recommendations for research

In comparison with many areas of research, investigation of chemoprevention is at an early stage. Not surprisingly, therefore, the research needs with regard to NSAIDs are similar to those for other types of chemopreventive agents. The current evidence is strong enough to indicate that NSAIDs as a group have properties that make them potentially important chemotherapeutic agents; however, there are significant gaps in knowledge, which are summarized below.

Most studies in experimental animals have been conducted with varying protocols and doses and have involved various agents. As a consequence, firm evidence for the relative potency of individual NSAIDs is lacking. In tissue culture, unrealistically high concentrations of NSAIDs have often been used to achieve effects, and in experimental animals attempts have not always been made to match the drug levels to those that might be attained in humans. There is a general deficiency of evidence about the levels achieved, whether in plasma, target tissues or colorectal tumours. Information on the levels achieved will be particularly important when the xenobiotic in question undergoes significant enterohepatic circulation. Finally, individual biomarkers such as cell proliferation as a measure of apoptosis or proliferation have not been applied consistently as surrogate end-points.

In epidemiological studies, there is a general lack of evidence on which to base an evaluation of the possible beneficial effects of individual non-aspirin NSAIDs. Such evidence may be obtainable within existing databases which hold information on drug usage and clinical outcomes, such as Medicard in the USA and the General Practitioner Research Database in the United Kingdom. Secondly, there is a relative lack of information on the possible benefits of NSAIDs against cancers outside the colon and rectum; again, this may be obtainable within the existing databases.

Clinical trials of cancer-preventive agents necessarily take many years to achieve results, and it is important that they be well designed and executed. In order to enhance quality and to minimize duplication of effort, it would be sensible to maintain a register of current studies, including sufficient detail for a realistic evaluation.

One feature of trials is likely to be the inclusion of aims to minimize drug toxicity. This design feature may require factorial methods and the inclusion of other promising chemopreventive agents, such as vitamin D and its analogues, in the hope that multiplicative actions can be obtained with reduced general toxicity. It is also noteworthy that slow-release NSAID preparations, which might reduce the general toxicity and delivery of the drug to the colon, could be of special value, although evidence of reduced gastric toxicity is limited.

Similar questions about the specificity, sensitivity and positive predictive value of surrogate end-points and biomarkers arise in clinical trials and experimental studies.

Randomized trials have been acknowledged as the means of providing unequivocal information about the efficacy of chemopreventive agents. The design of further trials of NSAIDs will be

influenced greatly by experimental indications of which agents are best subject to evaluation in this way. At the same time, it is becoming evident that the extended period required for certain chemopreventive agents to exert an effect makes study by randomized trial awkward. Observational studies may be the only adequate vehicle for such long-term investigations in low-risk populations.

6. Procedures used

In general, the content of the chapters in this Handbook is as indicated in the Preamble. The only known use of these agents is as pharmaceutical drugs. Accordingly, 'Use' is covered in Section 2.3, and Section 2.5 is concerned only with exposure as a consequence of taking the drug. The sections on 'Other beneficial effects' address the action of these drugs on cardiovascular health, and no attempt was made to summarize findings on the anti-inflammatory, antipyretic or analgesic effects of these agents.

A limited number of studies are reasonably characterized as involving use of NSAIDs for treatment, rather than prevention, of malignant disease, e.g. administration of a drug to patients with malignant gastric cancer. Such studies were not included.

The definition of 'sufficient evidence of cancer-preventive activity' in humans caused concern to members of the Working Group, because the level of certainty that a causal relationship has been established between the use of an agent and the prevention of human cancer must be higher for chemoprevention than for treatment in order to justify the use of an agent. A particular difficulty that the Working Group faced was to determine whether the available studies sufficed to rule out chance, bias or confounding with reasonable confidence, in the absence of long-term randomized trials specifically designed to test for putative cancer-preventive activity. The underlying problem is that administration of an agent to healthy people in order to prevent a relatively rare event will result in many opportunities for harm that substantially outweigh the benefits. Although this difficult is addressed in the overall evaluation of each agent, it necessarily influenced members of the Working Group in reaching their conclusions about the weight of the evidence.

7. References

Almoguera, C., Shibata, D., Forrester, K., Martin, J., Arnheim, N. & Perucho, M. (1988) Most human carcinomas of the exocrine pancreas contain mutant c-K-*ras* genes. *Cell,* **53**, 549–554

Ames, B.N., Shigenaga, M.K. & Hagen, T.M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. natl Acad. Sci. USA,* **90**, 7915–7922

Banerjee, A.K. (1989) Enteropathy induced by non-steroidal antiinflammatory drugs. Often subclinical, but may mimic Crohn's disease. *Br. med. J.,* **298**, 1539–1540

Basu, A.K. & Marnett, L.J. (1983) Unequivocal demonstration that malondialdehyde is a mutagen. *Carcinogenesis,* **4**, 331–333

Battellino, L.J., Dorronsoro de Cattoni, S.T. & Ragagnin, C. (1990) Impairment of human antipyrine metabolism by piroxicam. *Can. J. Physiol. Pharmacol.,* **68**, 711–717

Benamouzig, R., Chaussade, S., Little, J., Muñoz, N., Rantureau, J. & Couturier, D. (1997) [Aspirin, nonsteroidal anti-inflammatory agents and colon carcinogenesis.] *Gastroenterol. clin. biol.,* **21** (in press) (in French)

Bennett, A. & Del Tacca, M. (1975) Proceedings: Prostaglandins in human colonic carcinoma. *Gut,* **16**, 409

Bennett, A., Charlier, E.M., McDonald, A.M., Simpson, J.S., Stamford, I.F. & Zebro, T. (1977a) Prostaglandins and breast cancer. *Lancet,* **ii**, 624–626

Bennett, A., Tacca, M.D., Stamford, I.F. & Zebro, T. (1977b) Prostaglandins from tumours of human large bowel. *Br. J. Cancer,* **35**, 881–884

Bennett, A., Berstock, D.A., Raja, B. & Stamford, I.F. (1979) Survival time after surgery is inversely related to the amounts of prostaglandins extracted from human breast cancers. *Br. J. Pharmacol.,* **66**, 451P

Bennett, A., Civier, A., Hensby, C.N., Melhuish, P.B. & Stamford, I.F. (1987) Measurement of arachidonate and its metabolites extracted from human normal and malignant gastrointestinal tissues. *Gut.,* **28**, 315–318

Bhana, D., Hillier, K. & Karim, S.M. (1971) Vasoactive substances in Kaposi's sarcoma. *Cancer,* **27**, 233–237

Bilodeau, J.F., Wang, M., Chung, F.L. & Castonguay, A. (1995) Effecs of nonsteroidal antiinflammatory drugs on oxidative pathways in A/J mice. *Free Radical Biol. Med.*, 18, 47–54

Birkenfeld, S., Zaltsman, Y.A., Krispin, M., Zakut, H., Zor, U. & Kohen, F. (1987) Antitumor effects of inhibitors of arachidonic acid cascade on experimentally induced intestinal tumors. *Dis. Colon Rectum*, **30**, 43–46

Bjarnason, I., Zanelli, G., Smith, T., Prouse, P., Williams, P., Smethurst, P., Delacey, G., Gumpel, M.J. & Levi, A.J. (1987a) Nonsteroidal antiinflammatory drug-induced intestinal inflammation in humans. *Gastroenterology*, **93**, 480–489

Bjarnason, I., Zanelli, G., Prouse, P., Smethurst, P., Smith, T., Levi, S., Gumpel, M.J. & Levi, A.J. (1987b) Blood and protein loss via small intestinal inflammation induced by non-steroidal antiinflammatory drugs. *Lancet*, **ii**, 711–714

Bollini, P., Garcia Rodriguez, L.A., Perez Gutthann, S. & Walker, A.M. (1992) The impact of research quality and study design on epidemiologic estimates of the effect of nonsteroidal anti-inflammatory drugs on upper gastrointestinal tract disease. *Arch. intern. Med.*, **152**, 1289–1295

Borody, T.J., George, L.L., Brandl, S., Andrews, P., Ostapowicz, N., Hyland, L. & Devine, M. (1991) *Helicobacter pylori*-negative duodenal ulcer. *Am. J. Gastroenterol.*, **86**, 1154–1157

Borody, T.J., Brandl, S., Andrews, P., Jankiewicz, E. & Ostapowicz, N. (1992) *Helicobacter pylori*-negative gastric ulcer. *Am. J. Gastroenterol.*, **87**, 1403–1406

Botha, J.H., Robinson, K.M., Ramchurren, N., Reddi, K. & Norman, R.J. (1986) Human esophageal carcinoma cell lines: Prostaglandin production, biological properties, and behavior in nude mice. *J. natl Cancer Inst.*, **76**, 1053–1056

Boyd, J.A. & Eling, T.E. (1987) Prostaglandin H synthase-catalyzed metabolism and DNA binding by 2-naphthylamine. *Cancer Res.*, **47**, 4007–4014

Brée, F., Urien, S., Nguyen, P., Riant, P., Albengres, E. & Tillement, J.P. (1990) A re-evaluation of the HSA-piroxicam interaction. *Eur. J. Drug Metab. Pharmacokinet.*, **15**, 303–307

Brouwers, J.R. & de Smet, P.A. (1994) Pharmacokinetic–pharmacodynamic drug interactions with nonsteroidal anti-inflammatory drugs. *Clin. Pharmacokinet.*, **27**, 462–485

Burn, J., Chapman, P.D. & Eastham, E.J. (1994) Familial adenomatous polyposis. *Arch. Dis Child.*, **71**, 103–105

Combe, B., Edno, L., Lafforgue, P., Bologna, C., Bernard, J.C., Acquaviva, P., Sany, J. & Bressolle, F. (1995) Total and free methotrexate pharmacokinetics, with and without piroxicam, in rheumatoid arthritis patients. *Br. J. Rheumatol.*, **34**, 421–428

Craven, P.A., Saito, R. & DeRubertis, F.R. (1983) Role of local prostaglandin synthesis in the modulation of proliferative activity of rat colonic epithelium. *J. clin. Invest.*, **72**, 1365–1375

Cronen, P.W., Nagaraj, H.S., Janik, J.S., Groff, D.B., Passmore, J.C. & Hock, C.E. (1982) Effect of indomethacin on mesenteric circulation in mongrel dogs. *J. pediatr. Surg.*, **17**, 474–478

Cummings, K.B. & Robertson, R.P. (1977) Prostaglandin: Increased production by renal cell carcinoma. *J. Urol.*, **118**, 720–723

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kesmann, H., Ward, E. & Ryals, J. (1994) A central role of salicylic acid in plant disease resistance. *Science*, **266**, 1247

DeMello, M.C.F., Bayer, B.M. & Beaven, M.A. (1980) Evidence that prostaglandins do not have a role in the cytostatic action of anti-inflammatory drugs. *Biochem. Pharmacol.*, **29**, 311–318

DeWitt, D.L., Meade, E.A. & Smith, W.L. (1993) PGH synthase isoenzyme selectivity: The potential for safer nonsteroidal antiinflammatory drugs. *Am. J. Med.*, **95**, 40S–44S

Diczfalusy, U., Falardeau, P. & Hammarstrom, S. (1977) Conversion of prostaglandin endoperoxides to C-17 hydroxy acids catalyzed by human platelet thromboxane synthase. *FEBS Lett.*, **84**, 271–274

Dreyling, K.W., Hoppe, U., Peskar, B.A., Morgenroth, K., Kozuschek, W. & Peskar, B.M. (1986) Leukotriene synthesis by human gastrointestinal tissues. *Biochim. biophys. Acta*, **878**, 184–193

DuBois, R.N., Tsujii, M., Bishop, P., Awad, J.A., Makita, K. & Lanahan, A. (1994) Cloning and characterization of a growth factor-inducible cyclooxygenase gene from rat intestinal epithelial cells. *Am. J. Physiol.*, **266**, G822–G827

DuBois, R.N., Bishop, P.R., Graves-Deal, R. & Coffey, R.J. (1995) Transforming growth factor α regulation of the two zinc finger-containing immediate early response genes in intestine. *Cell Growth Differentiation*, **6**, 523–529

DuBois, R.N., Radhika, A., Reddy, B.S. & Entingh, A.J. (1996a) Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. *Gastro-enterology*, **110**, 1259–1262

DuBois, R.N., Shao, J.Y., Tsujii, M., Sheng, H. & Beauchamp, R.D. (1996b) G_1 delay in cells overexpressing prostaglandin endoperoxide synthase-2. *Cancer Res.*, **56**, 733–737

Eberhart, C.E., Coffey, R.J., Radhika, A., Giardiello, F.M., Ferrenbach, S. & DuBois, R.N. (1994) Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology*, **107**, 1183–1188

Ehsanullah, R.S.B., Page, M.C., Tildesly, G. & Wood, J.R. (1988) Prevention of gastroduodenal damage induced by non-steroidal anti-inflammatory drugs: Controlled trial of ranitidine. *Br. med. J.*, **297**, 1017–1021

El Attar, T.M. & Lin, H.S. (1987) Prostaglandin synthesis by squamous carcinoma cells of head and neck, and its inhibition by non-steroidal anti-inflammatory drugs. *J. oral Pathol.*, **16**, 483-487

Elder, D.J.E., Hague, A., Hicks, D.J. & Paraskeva, C. (1996) Differential growth inhibition by the aspirin metabolite salicylate in human colorectal tumor cell lines: Enhancedapoptosis in carcinoma and in vitro-transformed adenoma relative to adenoma cell lines. *Cancer Res.*, **56**, 2273–2276

Eling, T.E., Thompson, D.C., Foureman, G.L., Curtis, J.F. & Hughes, M.F. (1990) Prostaglandin H synthase and xenobiotic oxidation. *Ann. Rev. Pharmacol. Toxicol.*, **30**, 1–45

Elliott, S.L., Ferris, R.J., Giraud, A.S., Cook, G.A., Skerjo, M.V. & Yeomans, N.D. (1996) Indomethacin damage to rat gastric mucosa is markedly dependent on luminar pH. *Clin. exp. Pharmacol. Physiol.*, **23**, 432–434

Fazeli, A., Dickinson, S.L., Hermiston, M.L., Righe, R.V., Steen, R.G., Small, C.G., Stoeckli, E.T., Keino-Masu, K., Masu, M., Rayburn, H., Simons, J., Bronson, R.T., Gordon, J.I., Tessier-Lavigne, M. & Weinberg, R.A. (1997) Phenotype of mice lacking functional (deleted in colorectal cancer) DCC gene. *Nature*, **386**, 796–804

Fischer, A.R., Rosenberg, M.A., Lilly, C.M., Callery, J.C., Rubin, P., Cohn, J., White, M.V., Igarashi, Y., Kaliner, M.A., Drazen, J.M. *et al.* (1994) Direct evidence for a role of the mast cell in the nasal response to aspirin in aspirin-sensitive asthma. *J. Allergy clin. Immunol.*, **94**, 1046–1056

Fishel, R., Lescoe, M.K., Rao, M.R.S., Copeland, N.G., Jenkins, N.A., Garber, J., Kane, M. & Kolodner, R. (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell*, **75**, 1027–1038

Flower, R.J., Moncada, S., Vane, J.R., Gilman, A.G., Goodman, L.S., Rall, J.W. & Murad, F. eds (1985) *The Pharmacological Basis of Therapeutics*, 7th Ed., New York, MacMillan Publishing Co., pp. 674–715

Forrester, K., Almoguera, C., Han, K., Grizzle, W.E. & Perucho, M. (1987) Detection of high incidence of *K-ras* oncogenes during human colon tumorigenesis. *Nature*, **327**, 298–303

Fries, J.F., Williams, C.A., Bloch, D.A. & Michel, B.A. (1991a) Nonsteroidal anti-inflammatory drug-associated gastropathy: Incidence and risk factor models. *Am. J. Med.*, **91**, 213–222

Fries, J.F., Williams, C.A. & Bloch, D.A. (1991b) The relative toxicity of nonsteroidal antiinflammatory drugs. *Arthritis Rheum.*, **34**, 1353–1360

Frölich, J.C. (1997) A classification of NSAIDs according to the relative inhibition of cyclooxygenase isoenzymes. *TIPS*, **18**, 30–34

Gabriel, S.E., Jaakkimainen, L. & Bombardier, C. (1991) Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs. A meta-analysis. *Ann. intern. Med.*, **115**, 787–796

Gaffney, G.R. & Williamson, H.E. (1979) Effect of indomethacin and meclofenamate on canine mesenteric and celiac blood flow. *Res. Commun. Chem. Pathol. Pharmacol.*, **25**, 165–168

Gaido, K.W. & Wierda, D. (1987) Suppression of bone marrow stromal cell function by benzene and hydroquinone is ameliorated by indomethacin. *Toxicol. appl. Pharmacol.*, **89**, 378–390

Gann, P.H., Manson, J.E., Glynn, R.J., Buring, J.E. & Hennekens, C.H. (1993) Low dose aspirin and incidence of colorectal tumors in a randomized trial. *J. natl Cancer Inst.*, **85**, 1220–1224

Garcia Rodriguez, L.A. & Jick, H. (1994) Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs [published erratum appears in *Lancet* 1994, 343, 1048]. *Lancet,* **343**, 769–772

Garcia Rodriguez, L.A., Walker, A.M. & Perez Gutthann, S. (1992) Nonsteroidal antiinflammatory drugs and gastrointestinal hospitalizations in Saskatchewan: A cohort study. *Epidemiology,* **3**, 337–342

Garcia Rodriguez, L.A., Williams, R., Derby, L.E., Dean, A.D. & Jick, H. (1994) Acute liver injury associated with nonsteroidal anti-inflammatory drugs and the role of risk factors. *Arch. intern. Med.,* **154**, 311–316

Ghosh, J. & Myers, C.E. Jr (1996) Modulation of prostate cancer cell growth and invasion by arachidonic acid: Involvement of the metabolites of 5 lipoxygenase pathway (Abstract). *Proc. Am. Assoc. Cancer Res.*, **37**, 4111

Gierse, J.K., Hauser, S.D., Creely, D.P., Koboldt, C., Rangwala, S.H., Isakson, P.C. & Seibert, K. (1995) Expression and selective inhibition of the constitutive and inducible forms of human cyclooxygenase. *Biochem. J.,* **305**, 479–484

Gordon, L.I. & Weitzman, S.A. (1993) Inflammation and cancer. *Cancer J.,* **6**, 257–261

Graham, D.Y., Lidsky, M.D., Cox, A.M., Evans, D.J., Jr., Evans, D.G., Alpert, L., Klein, P.D., Sessoms, S.L., Michaletz, P.A. & Saeed, Z.A. (1991) Long-term nonsteroidal antiinflammatory drug use and *Helicobacter pylori* infection. *Gastroenterology,* **100**, 1653–1657

Greenberg, E.R., Baron, J.A., Freeman, T.H., Mandel, J.S. & Haile, R. (1993) Reduced risk of large bowel adenomas among aspirin users. *J. natl Cancer Inst.,* **85**, 912–916

Graves, E.J. & Kozak, L.J. (1992) National Hospital Discharge Survey. *Vital Health Stat.,* **13**, 1–51

Gridley, G., McLaughlin, J.K., Ekbom, A., Klareskog, L., Adami, H.O., Hacker, D.G., Hoover, R. & Fraumeni, J.F. (1993) Incidence of cancer among patients with rheumatoid arthritis. *J. natl. Cancer Inst.,* **85**, 307–311

Griffin, M.R., Ray, W.A. & Schaffner, W. (1988) Nonsteroidal anti-inflammatory drug use and death from peptic ulcer in elderly persons. *Ann. intern. Med.,* **109**, 359–363

Griffin, M.R., Piper, J.M., Daugherty, J.R., Snowden, M. & Ray, W.A. (1991) Nonsteroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elderly persons. *Ann. intern. Med.,* **114**, 257–263

Guess, H.A., West, R., Strand, L.M., Helston, D., Lydick, E.G., Bergman, U. & Wolski, K. (1988) Fatal upper gastrointestinal hemorrhage or perforation among users and nonusers of nonsteroidal anti-inflammatory drugs in Saskatchewan, Canada 1983. *J. clin. Epidemiol.,* **41**, 35–45

Gustafson-Svärd, C., Lilja, I., Hallbook, O. & Sjödahl, R. (1996) Cyclooxygenase-1 and cyclooxygenase-2 gene expression in human colorectal adenocarcinomas and in azoxymethane induced colonic tumours in rats. *Gut,* **38**, 79–84

Gutthann, S.P., Garcia Rodriguez, L.A. & Raiford, D.S. (1994) Individual non-steroidal anti-inflammatory drugs and the risk of hospitalization for upper gastrointestinal bleeding and perforation in Saskatchewan: A nested case–control study. *Pharmacoepidemiol. Drug Saf.,* **3**, S63

Hamberg, M. & Samuelsson, B. (1967) Oxygenation of unsaturated fatty acids by the vesicular gland of sheep. *J. biol. Chem.*, **242**, 5344–5354

Hanif, R., Pittas, A., Feng, Y., Koutsos, M.I., Qiao, L., Staiano-Coico, L., Shiff, S.I. & Rigas, B. (1996) Effects of nonsteroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway. *Biochem. Pharmacol.*, **52**, 237–245

Hecht, J.R., Rovai, L.E. & Herschman, H. (1995) Cyclooxygenase-2 expression and regulation in colonic mucosa, cancer and cell lines (Abstract). *Proc. Am. Assoc. Cancer Res.*, **36**, 598

Hendrickse, C.W., Kelly, R.W., Radley, S., Donovan, I.A., Keighley, M.R. & Neoptolemos, J.P. (1994) Lipid peroxidation and prostaglandins in colorectal cancer. *Br. J. Surg.,* **81**, 1219–1223

Henry, D. & Robertson, J. (1993) Nonsteroidal anti-inflammatory drugs and peptic ulcer hospitalization rates in New South Wales. *Gastroenterology,* **104**, 1083–1091

Henry, D., Dobson, A., Turner, C., Hall, P., Forbes, C. & Patey, P. (1991) NSAIDs and risk of upper gastrointestinal bleeding. *Lancet,* **337**, 730

Henry, D., Lim, L.L., Garcia Rodriguez, L.A., Perez Gutthann, S., Carson, J.L., Griffin, M., Savage, R., Logan, R., Moride, Y., Hawkey, C., Hill, S. & Fries, J.T. (1996) Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: Results of a collaborative meta-analysis. *Br. med. J.*, **312**, 1563–1566

Herschman, H.R. (1991) Primary response genes induced by growth factors and tumor promoters. *Ann. Rev. Biochem.*, **60**, 281–319

Hogan, D.B., Campbell, N.R., Crutcher, R., Jennett, P. & MacLeod, N. (1994) Prescription of nonsteroidal anti-inflammatory drugs for elderly people in Alberta. *Can. med. Assoc. J.*, **151**, 315–322

Honn, K.V., Bockman, R.S. & Marnett, L.J. (1981) Prostaglandins and cancer: A review of tumor initiation through tumor metastasis. *Prostaglandins*, **21**, 833–864

Hubbard, W.C., Alley, M.C., McLemore, T.L. & Boyd, M.R. (1988) Profiles of prostaglandin biosynthesis in sixteen established cell lines derived from human lung, colon, prostate, and ovarian tumors. *Cancer Res.*, **48**, 4770–4775

Hunt, M.D., Neuenschwander, U.H., Delaney, T.P., Weymann, K.B., Friedrich, L.B., Lawton, K.A., Steiner, H.Y. & Ryals, J.A. (1996) Recent advances in systemic acquired resistance research — A review. *Gene*, **179**, 89–95

Isomäki, H.A., Hakulinen, T. & Joutsenlahti, U. (1978) Excess risk of lymphomas, leukemia and myeloma in patients with rheumatoid arthritis. *J. chron. Dis.*, **31**, 691–696

Jaffe, B.M. (1974) Prostaglandins and cancer: An update. *Prostaglandins*, **6**, 453–461

Janssen, P.L. Hollman, P.C., Reichman, E., Venema, D.P., van Staveren, W.A. & Katan, M.B. (1996) Urinary salicylate excretion in subjects eating a variety of diets shows that amounts of bioavailable salicylates in foods are low. *Am. J. clin. Nutr.*, **64**, 743–747

Jass, J.R. & Stewart, J.M. (1992) Evolution of hereditary non-polyposis colorectal cancer. *Gut*, **33**, 783–786

Johnson, A.G., Seidemann, P. & Day, R.O. (1994a) NSAID-related adverse drug interactions with clinical relevance. An update. *Int. J. clin. Pharmacol. Ther.*, **32**, 509–532

Johnson, A.G., Nguyen, T.V. & Day, R.O. (1994b) Do nonsteroidal anti-inflammatory drugs affect blood pressure? A meta analysis. *Ann. intern. Med.*, **121**, 289–300

Johnson, A.G., Jick, S.S., Perera, D.R. & Jick, H. (1996) Histamine-2 receptor antagonists and gastric cancer. *Epidemiology*, **7**, 434–436

Kapui, Z., Boer, K., Rozsa, I., Blasko, G. & Hermecz, I. (1993) Investigations of indomethacin-induced gastric ulcer in rats. *Arzneimittel.-forsch.*, **43**, 767–771

Kargman, S.L., O'Neill, G.P., Vickers, P.J., Evans, J.F., Mancini, J.A. & Jothy, S. (1995) Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res.*, **55**, 2556–2559

Karmali, R.A. (1980) Review: Prostaglandins and cancer. *Prostaglandins Med.*, **5**, 11–18

Karmali, R.A. (1983) Prostaglandins and cancer. *CA Cancer J. Clin.*, **33**, 322–332

Kaufmann, H.J. & Taubin, H.L. (1987) Nonsteroidal anti-inflammatory drugs activate quiescent inflammatory bowel disease. *Am. J. Med.*, **107**, 513–516

Kinzler, K.W. & Vogelstein, B. (1996) Lessons from hereditary colon cancer. *Cell*, **87**, 159–170

Klapan, I., Katic, V., Culo, F. & Cuk, V. (1992) Prognostic significance of plasma prostaglandin E concentration in patients with head and neck cancer. *J. Cancer Res. clin. Oncol.*, **118**, 308–313

Koytchev, R., Alken, R.G. & Gromnica Ihle, E. (1994) Serum concentration of piroxicam and inhibition of platelet aggregation in patients with rheumatoid arthritis and M. Bechterew. *Agents Actions*, **43**, 48–52

Kudo, T., Narisawa, T. & Abo, S. (1980) Antitumor activity of indomethacin on methylzoxy-methanol-induced large bowel tumors in rats. *Gann*, **71**, 260–264

Kune, G.A., Kune, S. & Watson, L.F. (1988) Colorectal cancer risk, chronic illnesses, operations, and medications: Case control results from the Melbourne Colorectal Cancer Study. *Cancer Res.*, **48**, 4399–4404

Kutchera, W., Jones, D.A., Matsunami, N., Gordon, J., McIntyre, T.M., Zimmerman, G.A., White, R.L. & Prescott, S.M. (1996) Prostaglandin H synthase 2 is expressed abnormally in human colon cancer. Evidence for a transcriptional effect. *Proc. natl Acad. Sci. USA*, **93**, 4816–4820

Laakso, M., Mutru, O., Isomäki, H. & Koota, K. (1986) Cancer mortality in patients with rheumatoid arthritis. *J. Rheumatol.*, **13**, 522–526

Laneuville, O., Breuer, D.K., DeWitt, D.L., Hla, T., Funk, C.D. & Smith, W.L. (1994) Differential inhibition of human prostaglandin endoperoxide H synthases-1 and -2 by nonsteroidal anti-inflammatory drugs. *J. Pharmacol. exp. Ther.*, **271**, 927–934

Lange, K., Simmet, T., Peskar, B.M. & Peskar, B.A. (1985) Determination of 15-keto-13,14-dihydro-prostaglandin E2 and prostaglandin D2 in human colonic tissue using a chemiluminescence enzyme immunoassay with catalase as labeling enzyme. *Adv. Prostaglandin Thromboxane Leukotriene Res.*, **15**, 35–38

Langman, M.J., Morgan, L. & Worrall, A. (1985) Use of anti-inflammatory drugs by patients admitted with small or large bowel perforations and haemorrhage. *Br. med. J.*, **290**, 347–349

Langman, M.J., Weil, J., Wainwright, P., Lawson, D.H., Rawlins, M.D., Logan, R.F., Murphy, M., Vessey, M.P. & Colin Jones, D.G. (1994) Risks of bleeding peptic ulcer associated with individual non-steroidal anti-inflammatory drugs [published erratum appears in *Lancet*, 1994, **343**]. *Lancet*, **343**, 1075–1078

Lanza, L.L., Walker, A.M., Bortnichak, E.A. & Dreyer, N.A. (1995) Peptic ulcer and gastrointestinal hemorrhage associated with nonsteroidal anti-inflammatory drug use in patients younger than 65 years. A large health maintenance organization cohort study. *Arch. intern. Med.*, **155**, 1371–1377

Laporte, J.R., Carne, X., Vidal, X., Moreno, V. & Juan, J. (1991) Upper gastrointestinal bleeding in relation to previous use of analgesics and non-steroidal anti-inflammatory drugs. Catalan Countries Study on Upper Gastrointestinal Bleeding. *Lancet*, **337**, 85–89

Lewis, J.G., Stewart, W. & Adams, D.O. (1988) Role of oxygen radicals in induction of DNA by metabolites of benzene. *Cancer Res.*, **48**, 4762–4765

Ligumsky, M., Golanska, E.M., Hansen, D.G. & Kauffman, G.L. (1983) Aspirin can inhibit gastric mucosal cyclo-oxygenase without causing lesions in rat. *Gastroenterology*, **84**, 756–761

Lippmann, W. (1974) Inhibition of indomethacin-induced gastric ulceration in the rat by perorally-administered synthetic and natural prostaglandin analogues. *Prostaglandins*, **7**, 1–10

Lippton, H.L., Armstead, W.M., Hyman, A.L. & Kadowitz, P.J. (1987) Characterization of the vasoconstrictor activity of indomethacin in the mesenteric vascular bed of the cat. *Prostaglandins Leukotrienes Med.*, **27**, 81–91

Loeb, D.S., Talley, N.J., Ahlquist, D.A., Carpenter, H.A. & Zinsmeister, A.R. (1992) Long-term nonsteroidal anti-inflammatory drug use and gastroduodenal injury: The role of *Helicobacter pylori*. *Gastroenterology*, **102**, 1899–1905

Loll, P.J., Picot, D. & Garavito, R.M. (1995) The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H_2 synthase. *Nature Struct. Biol.*, **2**, 637–642

Loll, P.J., Picot, D., Ekabo, O. & Garavito, R.M. (1996) Synthesis and use of iodinated nonsteroidal antiinflammatory drug analogs as crystallographic probes of the prostaglandin H_2 synthase cyclooxygenase active site. *Biochemistry*, **35**, 7330–7340

Lu, X., Xie, W., Reed, D., Bradshaw, W.S. & Simmons, D.L. (1995) Nonsteroidal antiinflammatory drugs cause apoptosis and induce cyclooxygenases in chicken embryo fibroblasts. *Proc. natl Acad. Sci. USA*, **92**, 7961–7965

Lupulescu, A. (1975) Effect of prostaglandins on protein, RNA, DNA and collagen synthesis in experimental wounds. *Prostaglandins*, **10**, 573–579

Lupulescu, A.P. (1977) Cytologic and metabolic effects of prostaglandins on rat skin. *J. invest. Dermatol.*, **68**, 138–145

Lupulescu, A. (1978a) Enhancement of carcinogenesis by prostaglandins in male albino Swiss mice. *J. natl Cancer Inst.*, **61**, 97–106

Lupulescu, A. (1978b) Enhancement of carcinogenesis by prostaglandins. *Nature*, **272**, 634–636

Lupulescu, A. (1980) Heavy incorporation of 3H-prostaglandin F2 alpha in the neoplastic cells as revealed by autoradiographic studies. *Experentia*, **36**, 246–247

Marnett, L.J. (1990) Prostaglandin synthase-mediated metabolism of carcinogens and a potential role for peroxyl radicals as reactive intermediates. *Environ. Health Perspect.*, **88**, 5–12

Marnett, L.J. (1992) Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res.*, **52**, 5575–5589

Martin, D.F., Montgomery, E., Dobek, A.S., Patrissi, G.A. & Peura, D.A. (1989) *Campylobacter pylori*, NSAIDs, and smoking: Risk factors for peptic ulcer disease. *Am. J. Gastroenterol.*, **84**, 1268–1272

Marx, J. (1993) New colon gene discovered. *Science*, **260**, 751–752

Matsuyama, K., Sen, A.C. & Perrin, J.H. (1987) Binding of piroxicam to human serum albumin: Effect of piroxicam on warfarin and diazepam binding. *Pharm. Res.*, **4**, 355–357

Maxwell, W.J., Kelleher, D., Keating, J.J., Hogan, F.P., Bloomfield, F.J., MacDonald, G.S. & Keeling, P.W. (1990) Enhanced secretion of prostaglandin E2 by tissue-fixed macrophages in colonic carcinoma. *Digestion*, **47**, 160–166

McLellan, E.A., Medline, A. & Bird, R.P. (1991) Sequential analyses of the growth and morphological characteristics of aberrant crypt foci: Putative preneoplastic lesions. *Cancer Res.*, **51**, 5270–5274

Meade, E.A., Smith, W.L. & DeWitt, D.L. (1993). Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.*, **268**, 6610–6614

Mené, P., Pugliese, F. & Patrono, C. (1995) The effects of nonsteroidal anti-inflammatory drugs on human hypertensive vascular disease. *Semin. Nephrol.*, **15**, 244–252

Meyers, R.L., Alpan, G., Lin, E. & Clyman, R.I. (1991) Patent ductus arteriosus, indomethacin, and intestinal distension: Effects on intestinal blood flow and oxygen consumption. *Pediatr. Res.*, **29**, 569–574

Miller, T.A. (1983) Protective effects of prostaglandins against gastric mucosal damage: Current knowledge and proposed mechanisms. *Am. J. Physiol.*, **245**, G601–G623

Milligan, P.A., McGill, P.E., Howden, C.W., Kelman, A.W. & Whiting, B. (1993) The consequences of H2 receptor antagonist–piroxicam coadministration in patients with joint disorders. *Eur. J. clin. Pharmacol.*, **45**, 507–512

Mills, S.J., Chapman, P.D., Burn, J. & Gunn, A. (1997) Endoscopic screening and surgery for familial adenomatous polyposis: Dangerous delays. *Br. J. Surg.*, **84**, 774–777

Moorghen, M., Ince, P., Finney, K.J., Sunter, J.P., Appleton, D.R. & Watson, A.J. (1988) A protective effect of sulindac against chemically-induced primary colonic tumours in mice. *J. Pathol.*, **156**, 341–347

Moser, A.R., Pitot, H.C. & Dove, W.F. (1990) A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science*, **247**, 322–324

Moser, A.R., Mattes, E.M., Dove, W.F., Lindstrom, M.J., Haag, J.D. & Gould, M.N. (1993) Apc^{Min}, a mutation in the murine *Apc* gene, predisposes to mammary carcinomas and focal alveolar hyperplasias. *Proc. natl Acad. Sci. USA*, **90**, 8977–8981

Mostafa, M.H., Sheweita, S.A. & Abdel Moneam, N.M. (1990) Influence of some anti-inflammatory drugs on the activity of aryl hydrocarbon hydroxylase and the cytochrome P450 content. *Environ. Res.*, **52**, 77–82

Mukai, F.H. & Goldstein, B.D. (1976) Mutagenicity of malondialdehyde, a decomposition product of peroxidized polyunsaturated fatty acids. *Science*, **191**, 868–869

Mulder, C.J.J., Fockens, P., Meijer, J.W.R., van der Heide, H., Wiltink, Ed H.H. & Tygat, G. N.J. (1996) Beclomethasone dipropionate (3 mg) versus 5 amino-salicylic acid (2 g) versus the combination of both (3 mg/2 mg) as retention enemas in active ulcerative proctitis. *Eur. J. Gastro-hepatol.*, **8**, 549–553

Nakamura, A., Yamatani, T., Fujita, T. & Chiba, T. (1991) Mechanism of inhibitory action of prostaglandins on the growth of human gastric carcinoma cell line KATO III. *Gastroenterology*, **101**, 910–918

Narisawa, T., Sato, M., Tani, M., Kudo, T., Takahashi, T. & Goto, A. (1981) Inhibition of development of methylnitrosourea-induced rat colon tumors by indomethacin. *Cancer Res.*, **41**, 1954–1957

Narisawa, T., Satoh, M., Sano, M. & Takahashi, T. (1983) Inhibition of initiation and promotion by *N*-methylnitrosourea-induced colon carcinogenesis in rats by non-steroid and anti-inflammatory agent indomethacin. *Carcinogenesis*, **4**, 1225–1227

Narisawa, T., Hermanek, P., Habs, M. & Schmähl, D. (1984) Reduction of carcinogenicity of N-nitrosomethylurea by indomethacin and failure of resuming effect of prostaglandin E_2 (PGE_2) against indomethacin. *J. Cancer Res. clin. Oncol.*, **108**, 239–242

Narisawa, T., Kusaka, H., Yamazaki, Y., Takahashi, M., Koyama, H., Koyama, K., Fukaura, Y. & Wakizaka, A. (1990) Relationship between blood plasma prostaglandin E2 and liver and lung metastases in colorectal cancer. *Dis. Colon Rectum,* **33**, 840–845

Nathans, D., Lau, L.F., Christy, B., Hartzell, S., Nakabeppu, Y. & Ryder, K. (1988) Genomic response to growth factors. *Cold Spring Harbor Symp. Quant. Biol.,* **53**, 893–900

Needleman, P., Turk, J., Jakschik, B.A., Morrison, A.R. & Lefkowith, J.B. (1986) Arachidonic acid metabolism. *Ann. Rev. Biochem.,* **55**, 69–102

Nensey, Y.M., Schubert, T.T., Bologna, S.D. & Ma, C.K. (1991) *Helicobacter pylori*-negative duodenal ulcer. *Am. J. Med.,* **91**, 15–18

Ohki, S., Ogino, N., Yamamoto, S. & Hayashi, O. (1979) Prostaglandin hydro-peroxidase, an integral part of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. *J. biol. Chem.,* **254**, 829–836

Ohshima, H. & Bartsch, H. (1994) Chronic infections and inflammatory processes as cancer risk factors: Possible role of nitric oxide in carcinogenesis. *Mutat. Res.,* **305**, 253–264

O'Neill, G.P., Mancini, J.A., Kargman, S., Yergey, J., Kwan, M.Y., Falgueyret, J.P., Abramovitz, M., Kennedy, B.P., Ouellet, M., Cromlish, W. *et al.* (1994) Overexpression of human prostaglandin G/H synthase-1 and -2 by recombinant vaccinia virus: Inhibition by nonsteroidal anti-inflammatory drugs and biosynthesis of 15-hydroxy-eicosatetraenoic acid. *Mol. Pharmacol.,* **45**, 245–254

Ohshima, M., Dinchuk, J.E., Kargman, S., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J.M., Evans, J.F. & Taketo, M.M. (1996) Suppression of intestinal polyposis in *Apc*^{D761} knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell,* **87**, 803–809

Parkin, D.M., Pisani, P. & Ferlay, J. (1993) Estimates of worldwide incidence of eighteen major cancers in 1985. *Int. J. Cancer,* **54**, 594–606

Papadopoulos, N. Nicolaides, N.C., Wei, Y.F., Ruben, S.M., Carter, K.C., Rosen, C.A., Haseltine, W.A., Fleischmann, R.D., Fraser, C.M., Adams, M.D. *et al.* (1994) Mutation of *mutL* homolog in hereditary colon cancer. *Science,* **263**, 1625–1629

Picot, D., Loll, P.J. & Garavito, R.M. (1994) The X-ray crystal structure of the membrane protein prostaglandin H_2 synthase-1. *Nature,* **367**, 243–249

Piper, J.M., Ray, W.A., Daughety, J.R. & Griffin, M.R. (1991) Corticosteroid use and peptic ulcer disease: Role of nonsteroidal anti-inflammatory drugs. *Ann. intern. Med.,* **114**, 735–740

Pirozzi, S.J., Renz, J.F. & Kalf, G.F. (1989b) The prevention of benzene-induced genotoxicity in mice by indomethacin. *Mutat. Res.,* **222**, 291–298

Pirozzi, S.J., Schlosser, M.J. & Kalf, G.F. (1989) Prevention of benzene-induced myelotoxicity and prostaglandin synthesis in bone marrow of mice by inhibitors of prostaglandin H synthase. *Immunopharmacology,* **18**, 39–55

Pollard, M. & Luckert, P.H. (1980) Indomethacin treatment of rats with dimethylhydrazine-induced intestinal tumors. *Cancer Treat. Rep.,* **64**, 1323–1327

Pollard, M. & Luckert, P.H. (1981a) Effect of indomethacin on intestinal tumors induced in rats by the acetate derivative of dimethylnitrosamine. *Science,* **214**, 558–559

Pollard, M. & Luckert, P.H. (1981b) Treatment of chemically-induced intestinal cancers with indomethacin. *Proc. Soc. exp. Biol. Med.,* **167**, 161–164

Pollard, M. & Luckert, P.H. (1983) Prolonged antitumor effect of indomethacin on autochthonous intestinal tumors in rats. *J. natl Cancer Inst.,* **70**, 1103–1105

Pollard, M. & Luckert, P.H. (1984) Effect of piroxicam on primary intestinal tumors induced by rats by *N*-methylnitrosourea. *Cancer Lett.,* **25**, 117–121

Pollard, M., Luckert, P.H. & Schmidt, M.A. (1983) The suppressive effect of piroxicam on autochthonous intestinal tumors in the rat. *Cancer Lett.,* **21**, 57–61

Powell, S.M., Zilz, N., Beazer-Barclay, Y., Bryan, T.M., Hamilton, S.R., Thibodeau, S.N., Vogelstein, B. & Kinzler, K.W. (1992) *APC* mutations occur early during colorectal tumorigenesis. *Nature,* **359**, 235–237

Prescott, S.M. & White, R.L. (1996) Self-promotion? Intimate connections between APC and prostaglandin H synthase-2. *Cell,* **87**, 783–786

Pretlow, T.P., O'Riordan, M.A., Pretlow, T.G. & Stellato, T.A. (1992) Aberrant crypts in human colonic mucosa: Putative preneoplastic lesions. *J. Cell Biochem.* **16G**, 55–62X

Rampton, D.S. (1987) Non-steroidal anti-inflammatory drugs and lower gastrointestinal tract? *Scand. J. Gastroenterol.*, **22**, 1–4

Ravi, S., Keat, A.C. & Keat, E.C.B. (1986) Colitis caused by non-steroidal anti-inflammatory drugs. *Postgrad. med. J.*, **62**, 773–776

Rao, C.V., Tokumo, K., Rigotty, J., Zang, E., Kelloff, G. & Reddy, B.S. (1991) Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, α-difluoromethylornithine, 16α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. *Cancer Res.*, **51**, 4528–4534

Rao, C.V., Desai, D., Simi, B., Kulkarni, N., Amin, S. & Reddy, B.S. (1993) Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon. *Cancer Res.*, **53**, 4182–4188

Reddy, B.S., Maruyama, H. & Kelloff, G. (1987) Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflammatory drug, during different stages of rat colon tumor development. *Cancer Res.*, **47**, 5340–5346

Reddy, B.S., Nayini, J., Tokumo, K., Rigotty, J., Zang, E. & Kelloff, G. (1990) Chemoprevention of colon carcinogenesis by concurrent administration of piroxicam, a nonsteroidal antiinflammatory drug with D,L-α-difluoromethylornithine, an ornithine decarboxylase inhibitor, in diet. *Cancer Res.*, **50**, 2562–2568

Reddy, B.S., Tokumo, K., Kulkarni, N., Aligia, C. & Kelloff, G. (1992) Inhibition of colon carcinogenesis by prostaglandin synthesis inhibitors and related compounds. *Carcinogenesis*, **13**, 1019–1023

Reddy, B.S., Rao, C.V. & Seibert, K. (1996) Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. *Cancer Res.*, **56**, 4566–4569

Reed, G.A. (1988) Oxidation of environmental carcinogens by prostaglandin H synthase. *J. environ. Sci. Health*, **C6**, 223–259

Rice, J.R., Zenser, T.V. & Davis, B.B. (1985) Prostaglandin synthase-dependent cooxidation and aromatic amine carcinogenesis. *Prostaglandins Leukotrienes Cancer*, **2**, 125–129

Rigas, B., Goldman, I.S. & Levine, L. (1993) Altered eicosanoid levels in human colon cancer. *J. Lab. clin. Med.*, **122**, 518–523

Rioux, K.P. & Wallace, J.L. (1996) Mast cells do not contribute to nonsteroidal anti-inflammatory drug-induced gastric mucosal injury in rodents. *Aliment. pharmacol. Ther.*, **10**, 173–180

Rodenhuis, S. & Slebos, R.J. (1992) Clinical significance of *ras* oncogene activation in human lung cancer. *Cancer Res.*, **52**, 2665s–2669s

Rolland, P.H., Martin, P.M., Jacquemier, J., Rolland, A.M. & Toga, M. (1980) Prostaglandin in human breast cancer: Evidence suggesting that an elevated prostaglandin production is a marker of high metastatic potential for neoplastic cells. *J. natl Cancer Inst.*, **64**, 1061–1070

Said, S.A. & Foda, A.M. (1989) Influence of cimetidine on the pharmacokinetics of piroxicam in rat and man. *Arzneimittel.-forsch.*, **39**, 790–792

Salvemini, D., Misko, T.P., Masferrer, J.L., Seibert, K., Currie, M.G. & Needleman, P. (1993) Nitric oxide activates cyclooxygenase enzymes. *Proc. natl Acad. Sci. USA*, **90**, 7240–7244

Sandler, M., Karim, S.M. & Williams, E.D. (1968) Prostaglandins in amine-peptide-secreting tumours. *Lancet*, **ii**, 1053–1054

Sano, H., Kawahito, Y., Wilder, R.L., Hashiramoto, A., Mukai, S., Asai, K., Kimura, S., Kato, H., Kondo, M. & Hla, T. (1995) Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res.*, **55**, 3785–3789

Santoro, M., Philpott, G.W. & Jaffe, B.M. (1976) Inhibition of tumour growth in vivo and in vitro by prostaglandin E. *Nature*, **263**, 777–779

Savage, R.L., Moller, P.W., Ballantyne, C.L. & Wells, J.E. (1993) Variation in the risk of peptic ulcer complications with nonsteroidal antiinflammatory drug therapy. *Arthr. Rheum.*, **36**, 84–90

Scheiman, J.M., Behler, E.M., Loeffler, K.M & Elta, G.H. (1994) Omeprazole ameliorates aspirin-induced gastroduodenal injury. *Dig. Dis. Sci.*, **39**, 97–103

Schlosser, M.J., Shurina, R.D. & Kalf, G.F. (1989) Metabolism of phenol and hydroquinone to reactive products by macrophage peroxidase or purified prostaglandin H synthase. *Environ. Health Perspect.*, **82**, 229–237

Schoon, I.M., Mellstrom, D., Oden, A. & Ytterberg, B.O. (1989) Incidence of peptic ulcer disease in Gothenburg, 1985 [published erratum appears in *Br. med. J.*, 1990, **301**, 906]. *Br. med. J.*, **299**, 1131–1134

Shimakura, S. & Boland, S.R. (1992) Eicosanoid production by the human gastric cancer cell line AGS and its relation to cell growth. *Cancer Res.*, **52**, 1744–1749

Shorr, R.I., Ray, W.A., Daugherty, J.R. & Griffin, M.R. (1993) Concurrent use of nonsteroidal anti-inflammatory drugs and oral anticoagulants places elderly persons at high risk for hemorrhagic peptic ulcer disease. *Intern. Med.*, **153**, 1665–1670

Silverstein, F.E., Graham, D.Y., Senior, J.Y., Davies, H.W., Struthers, B.J., Bittman, R.M. & Geis, G.S. (1995) Misoprostil reduces serious gastrointestinal complications in patients with rheumatoid arthritis receiving nonsteroidal anti-inflammatory drugs. A randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med.*, **123**, 241–249

Smalley, W.E., Ray, W.A., Daugherty, J.R. & Griffin, M.R. (1995) Nonsteroidal anti-inflammatory drugs and the incidence of hospitalizations for peptic ulcer disease in elderly persons. *Am. J. Epidemiol.*, **141**, 539–545

Smalley, W.E., Griffin, M.R., Fought, R.L. & Ray, W.A. (1996) Excess costs for gastrointestinal disease among nonsteroidal anti-inflammatory drug users. *J. gen. intern. Med.*, **11**, 461–469

Smith, B.J., Curtis, J.F. & Eling, T.E. (1991) Bioactivation of xenobiotics by prostaglandin H synthase. *Chem.- biol. Interact.*, **79**, 245–264

Somerville, K., Faulkner, G. & Langman, M. (1986) Non-steroidal anti-inflammatory drugs and bleeding peptic ulcer. *Lancet*, **i**, 462–464

Spalding, J.W. (1988) Toxicology and carcinogenesis studies of malondialdehyde sodium salt (3-hydroxy-2-propenal, sodium salt) in F/344 N rats and B6C3F1 mice. *NTP tech. Rep.*, **331**, 5–13

Staton, G.W.J., Ingram, R.H., Dale, D.C. & Federman, D.D., eds (1996) *Asthma*, New York, Scientific American Inc., pp. 9–10

Steering Committee of the Physicians' Health Study Research Group (1989) Final report on the aspirin component of the ongoing Physicians' Health Study. *New Engl. J. Med.*, **321**, 129–135

Su, L.-K., Kinzler, K.W., Vogelstein, B., Preisinger, A.C., Moser, A.R., Luongo, C., Gould, K.A. & Dove, W.F. (1992) Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science*, **256**, 668–670

Suh, O., Mettlin, C. & Petrelli, N.J. (1993) Aspirin use, cancer and polyps of the large bowel. *Cancer*, **73**, 1171–1177

Szczeklik, A. (1994) Aspirin-induced asthma: An update and novel findings. *Adv. Prostaglandin Thromboxane Leukotriene Res.*, **22**, 185–198

Taha, A.S., Hudson, N.J., Hawkey, C.J., Swannell, A.J., Trye, P.N., Cottrell, J., Mann, S.G., Simon, T.J., Sturrock, B.R. & Russell, R.I. (1996) Famotidine for the prevention of gastric and duodenal ulcers caused by nonsteroidal anti-inflammatory drugs. *New Engl. J. Med.*, **334**, 1435–1439

Takeuchi, K., Takehara, K. & Ohuchi, T. (1996) Diethyldithiocarbamate, a superoxide dismutase inhibitor, reduces indomethacin induced gastric lesions in rats. *Digestion*, **57**, 201–209

Thun, M.J., Namboodiri, M.N., Calle, E.E., Flanders, W.D. & Heath, C.W. (1993) Aspirin use and risk of fatal cancer. *Cancer Res.*, **53**, 1322–1327

Tsujii, M. & DuBois, R.N. (1995) Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell*, **83**, 493–501

Tutton, P.J. & Barkla, D.H. (1980) Influence of prostaglandin analogues on epithelial cell proliferation and xenograft growth. *Br J. Cancer*, **41**, 47–51

Van Der Ouderaa, F.J., Buytenhek, M., Nugteren, D.H. & Van Dorp, D.A. (1980) Acetylation of prostaglandin endoperoxide synthetase with acetylsalicylic acid. *Eur. J. Biochem.*, **109**, 1–8

Vane, J.R. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature new Biol.*, **231**, 232–235

Vane, J.R., Flower, R.J. & Botting, R.M. (1990) History of aspirin and its mechanism of action. *Stroke*, **21**(Suppl. 4), 12–23

Verbeeck, R.K. (1990) Pharmacokinetic drug interactions with nonsteroidal anti-inflammatory drugs. *Clin. Pharmacokinet.*, **19**, 44–66

Vogelstein, B. & Kinzler, K.W. (1993) The multistep nature of cancer. *Trends Genet.*, **9**, 138–141

Vogelstein, B. & Kinzler, K.W. (1994) Colorectal cancer and the intersection between basic and clinical research. *Cold Spring Harbor Symp. Quant. Biol.*, **59**, 517–521

Wallace, J.L. (1997) Nonsteroidal anti-inflammatory drugs and gastroenteropathy. The second hundred years. *Gastroenterology*, **112**, 1000–1016

Wargovich, M.J., Chen, C.D., Harris, C., Yang, E. & Velasco, M. (1995) Inhibition of aberrant crypt growth by non-steroidal anti-inflammatory agents and differentiation agents in the rat colon. *Int. J. Cancer*, **60**, 515–519

Watson, S.A., Clifford, T., Sykes, R.E., Robinson, E. & Steele, R.J.C. (1995) Gastrin sensitivity of primary human colorectal cancer: The effect of gastrin receptor antagonism. *Eur. J. Cancer*, **31**A, 2086–2092

Williams, C.S. & DuBois, R.N. (1996) Prostaglandin endoperoxide synthase: Why two isoforms? *Am. J. Physiol.*, **270**, 393–400

Williams, E.D., Karim, S.M. & Sandler, M. (1968) Prostaglandin secretion by medullary carcinoma of the thyroid. A possible cause of the associated diarrhoea. *Lancet*, **i**, 22–23

Williams, C.W., Luongo, C., Radhika, A., Zhang, T., Lamps, L.W., Nanney, L.B., Beauchamp, R.D. & DuBois, R.N. (1996) Elevated cyclooxygenase-2 levels in Min mouse adenomas. *Gastroenterology*, **110**, 1134–1140

Wright, V. (1993). Historical overview of NSAIDs. *Eur. J. Rheum. Inflamm.*, **13**, 4–6

Yoshikawa, T., Naito, Y., Kishi, A., Tomii, T., Kaneko, T., Linuma, S., Ichikawa, H., Yasucda, M., Takahashi, S. & Kondo, M. (1993) Role of active oxygen, lipid peroxidation, and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. *Gut*, **34**, 732–737

Zahavi, I., Fisher, S., Marcus, H., Heckelman, B., Kiro, A. & Dinari, G. (1995) Oxygen radical scavengers are protective against indomethacin-induced intestinal ulceration in the rat. *J. Pediatr. Gastroenterol. Nutr.*, **21**, 154–157