Genotoxic and epigenetic chemical carcinogenesis: one process, different mechanisms

W. K. Lutz and P. Maier

Chemicals that induce cancer in an intact organism are called carcinogens. This term does not differentiate between their various modes of action. In this review, Werner Lutz and Peter Maier *make a mechanistic distinction between carcinogens that alter the genetic information and carcinogens that interfere with epigenetic processes. They consider carcinogenesis to be an ongoing, partly unavoidable process which is based on a succession of mutations, most likely in stem cells, leading to autonomous cellular growth regulation. Chemical carcinogens either induce such changes through mutations (genotoxic carcinogens) or they accelerate the accumulation of critical spontaneous mutations (epigenetic carcinogens). Examples are given for both classes of carcinogens, and for the processes that act at genotoxic/nuclear and epigenetic/mitotic levels.*

Cancer cells differ from normal cells by their autonomous and invasive growth in a tissue. This phenotype is a consequence of irreversible, heritable changes in structure or expression of the genetic material. During the last few years, a number of genes have been identified in mammalian cells that seem to be involved in the process of neoplastic cell transformation. These genes, which when active can confer on a cell features of a cancerous phenotype, are termed proto-oncogenes. Many of these gene products are related to pathways that determine the cell's response to growth-stimulating factors and/or differentiation, e.g. growth factors, growth factor receptors, cytoplasmic and nuclear regulatory proteins[1]. In normal cells, their expression is controlled by the specific requirements for growth and differentiation.

Conversion of the proto-oncogenes into oncogenes (activation) by genetic events, such as base-pair substitution or translocations of the gene into actively transcribed regions of the genome by chromosomal rearrangements[2], are the best studied mechanisms. These mutations result in the expression of abnormal gene product, the deregulated expression of a proto-oncogene at the wrong time during ontogenesis of a cell, or the expression of an abundance of the proto-oncogene product. The mutations involved are characteristic for the specific tissue and cause autonomous clonal growth of tumour cells.

Alternatively, oncogene expression can be modified by the loss or inhibition of functions encoded by regulatory genes, the so-called tumour suppressor genes[3]. Suppressor genes probably encode DNA-binding proteins that inhibit the transcription of oncogenes or prevent the expression of the tumourigenic phenotype itself. This mechanism was proposed to play a role in familial predisposition to cancer (e.g. retinoblastoma)[4]. The cancer-causing gene can be present as a recessive trait and will be expressed when the other normal gene, which acts as a suppressor gene, is functionally lost. This can occur in a clone of somatic cells as a result of a number of chromosomal mechanisms, such as non-disjunction, mitotic recombination, gene conversion, or small deletions[5]. A change in the genetically controlled pattern of cytosine methylation in the DNA is a further mechanism which might be responsible for permanent oncogene expression.

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Carcinogenesis as a multi-stage process

Some age-specific cancer incidence rates rise exponentially with age. Analysis of this time dependence reveals that several distinct stages are involved in tumour growth (Fig. 1). The number of stages may be around five to seven for most sites[6], and it is likely that for some childhood cancer types (e.g. leukaemia, brain) the process begins *in utero*. *In vitro*, at least two oncogenes have to be expressed in order for a primary cell to be transformed[7]. Different types of mutations or a combination of them might be necessary at each stage of carcinogenesis.

Initiation of carcinogenesis by chemical mutagens

Chemicals that can interact with the genetic material and generate critical DNA lesions are called tumour initiating agents or genotoxic carcinogens. Most of those identified so far cause gene mutations, but this is probably due to the fact that this type of mutation is detectable efficiently by relatively simple microbial test systems. In fact, genotoxic chemicals often induce wide spectra of mutations. Evidence exists that specific chemical mutations can occur in codons of cellular proto-oncogenes, as was shown with the c-Ha-*ras* gene, using nitrosomethylurea[8] or dimethylbenzanthracene[9]. However, the appearance of specific aberrant chromosomes in tumour cells[2] suggests that induced chromosomal rearrangements and deletions have an equal or greater significance as initial events.

Direct interaction with DNA

The best studied interaction involves covalent binding of the carcinogen to nuclear DNA[10], either spontaneously (alkylating agents such as epoxides, mustards, alkyl sulphonates or alkyl halides) or after enzymatic activation to an electrophilic metabolite. This is the mechanism of most well known potent chemical carcinogens, e.g. the polycyclic aromatic hydrocarbons (benzo[*a*]pyrene), aromatic amines (2-naphthylamine), *N*-nitroso compounds (*N*-nitrosodimethylamine), and compounds containing an olefin with substituents that render it electrophilic as in aflatoxins or vinyl chloride. If the resulting DNA adduct is

not removed and the lesion correctly repaired, gene mutations and, in rare cases, chromosome aberrations can arise following DNA replication. Mutations induced in the mitochondrial DNA, especially in combination with mutations in the nucleus, might also play a role in carcinogenesis[11].

Alteration of chromosome structure

Besides covalent DNA binding of the compound or one of its metabolites, DNA lesions can be induced by free radicals, as in the case of radiation. The resulting mutation will predominantly be due to chromosome breakage (rearrangements, deletions). One hypothesis is that some carcinogens exert their effect by generating reactive oxygen species (including the hydroxyl radical) which in turn damage DNA or DNA–protein complexes. This mechanism has been postulated for a number of cellular peroxidant states[12].

The list of compounds includes those hepatic carcinogens that induce a proliferation of peroxisomes [phthalate plasticizers like di(2-ethylhexyl)phthalate or hypolipidaemic agents such as clofibrate]. Although this is an appealing hypothesis we are not aware of any report that demonstrates DNA damage in a target tissue after treatment of animals with a peroxisome proliferator.

Interference with DNA replication

The process of DNA replication can be disturbed, for example, by certain metal ions (such as Be^{2+}, Cd^{2+}, Ni^{2+}) which can reduce template fidelity, or by promoting the incorporation of mis-matching nucleotides, or by the disturbance of the nucleotide pool with base analogues and inhibitors of DNA-precursor synthesis (e.g. fluorouracil, methothrexate). These interactions again predominantly result in structural chromosome aberrations[13].

Interference with DNA segregation

Agents that induce numerical chromosomal aberrations [e.g. spindle poisons like demecolcine (colcemid)] could also lead to an enhanced expression of oncogene products. This can be achieved by increasing the copies of genes per cell (gene amplification, hyperploidy) or by activation due to a hemizygotic gene constellation involving suppressor genes (hypoploidy). However, numerical chromosome aberrations often impair normal cell growth and are unlikely to play an important role in the early steps of carcinogenesis.

Interaction with DNA metabolism in damaged cells

It is important to realize that high doses of genotoxic agents induce local, often undetected, cytotoxicity in target tissue (necrosis). This might have happened in those experiments in which carcinogenicity of chemicals was demonstrated at highest exposure levels only. From *in-vitro* mutagenicity studies it is known that unphysiological conditions alone cause an increase in the number of

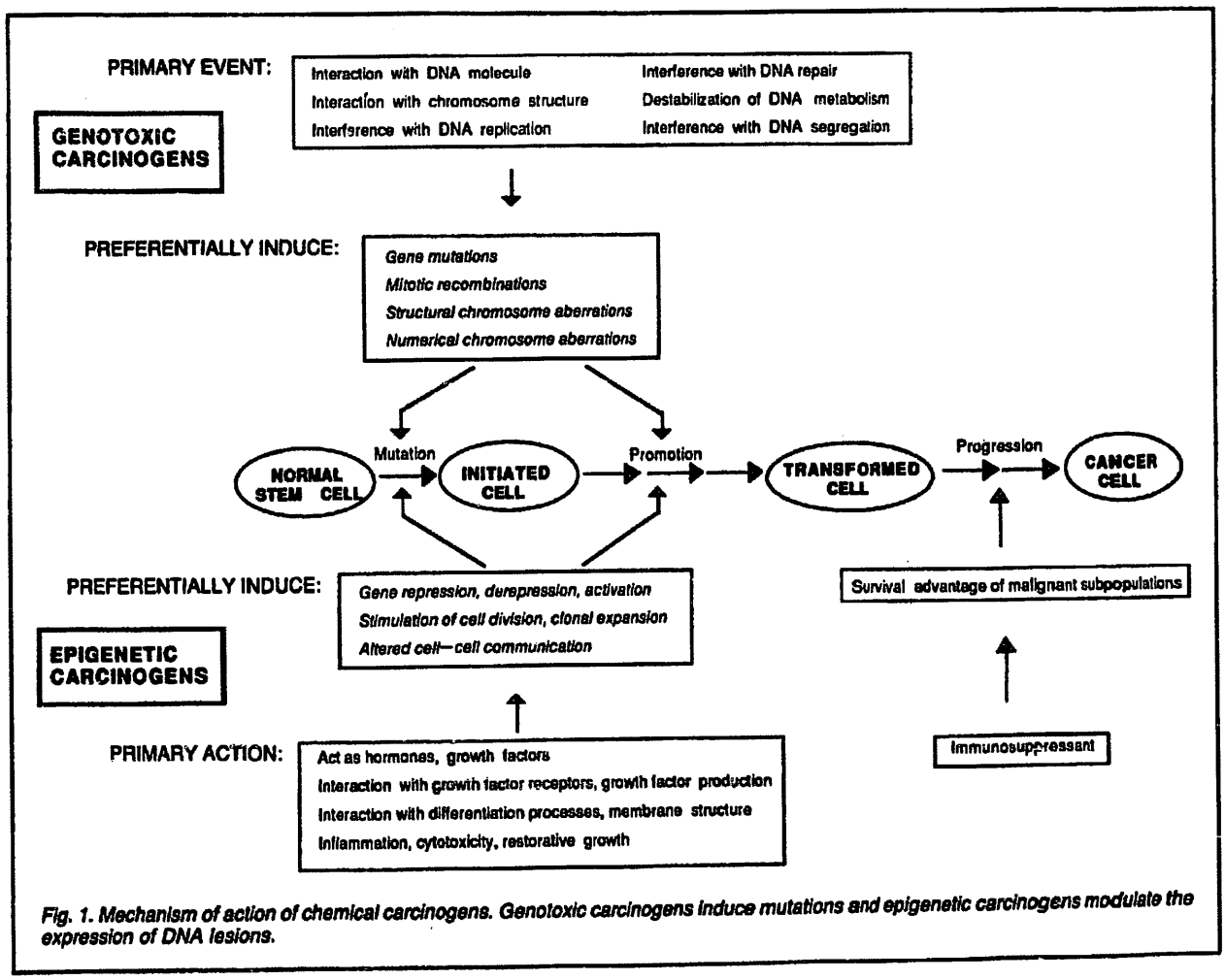


Fig. 1. Mechanism of action of chemical carcinogens. Genotoxic carcinogens induce mutations and epigenetic carcinogens modulate the expression of DNA lesions.

mutations[14]. It cannot be ruled out that *in vivo* as well, in heavily damaged but surviving and proliferating cells, errors accumulate in pathways that are involved in the maintenance of an intact genome.

Carcinogenesis as an ongoing, partly unavoidable process

Animals fed on a controlled diet free of known potent carcinogens also develop cancer. This background rate of tumour incidence is called 'spontaneous' and indicates that all requirements for cancer induction are met in the absence of added carcinogen. Carcinogenesis therefore must, to a degree, be considered an unavoidable process.

Spontaneous DNA damage results from intrinsic DNA instability, and from endogenous electrophiles and radicals. The first aspect includes spontaneous deaminations of adenine and cytosine and depurinations, as well as base mispairings due to tautomer formation and the presence of mutagenic metal ions.

The list of endogenous electrophiles includes *S*-adenosylmethionine, aldehydes (e.g. sugars, formaldehyde), epoxide intermediates in the oxidation of aromatic amino acid derivatives and steroids, and quinones (e.g. from estrogens, catecholamines). The formation of radicals is also unavoidable because of the continuous leakage of the superoxide anion radical after the first reduction step of molecular oxygen by various cytochromes. Although protective enzyme systems (superoxide dismutase and peroxidases) have evolved for the protection of cellular components, a minor fraction will inevitably form the highly reactive hydroxyl radical ·OH in the presence of catalytic concentrations of metal ions, especially iron.

In addition to endogenous sources of DNA damage, there are also unavoidable environmental factors. The most important ones are probably ionizing radiation arising from the decay of terrestrial radioactive material, including inhaled radon, and from cosmic rays, including ultraviolet light.

It must therefore be concluded that a low level of DNA damage cannot be avoided. This conclusion is supported by the recent detection of age-related DNA modifications in untreated rats[15] and the identification of activated proto-oncogenes in spontaneous hepatocellular tumours in mice[16]. In germ cells, these modifications may play an important role in evolutionary processes. However, in somatic cells, they inevitably form the genotoxic basis for so-called spontaneous tumour formation. Therefore, the introduction of the concept of an endogenous basis for all steps required for carcinogenesis allows the hazard from exposure to an exogenous chemical carcinogen to be estimated in terms of an increment only.

Tumour promotion

The time in humans from the first critical mutation to the expression of a malignant tumour is between a few years (leukaemia from ionizing radiation) to more than two decades (lung cancer from smoking). This period is subject to modulation by endogenous and exogenous, genetic and/or epigenetic factors.

In studies on the role of inflammation in carcinogenesis, it was found that certain compounds (phorbol esters in croton resin in the early experiments), when painted over a long period of time on the skin of mice, dramatically increased skin tumour formation after a single administration of a genotoxic carcinogen[17]. The early effect of the genotoxic compound was later termed initiation, while the long-term treatment was called tumour promotion. Treatment with the tumour promoter alone did not lead to a significant increase in the number of tumours in these early experiments. Also, since the tumour promoters were not mutagenic in most short-term assays for mutagenicity, they were not classified as carcinogens.

However, latterly it has been discovered that tumour promoters alone can increase cancer incidence if tested on large numbers of animals over their entire life span. It must be assumed that under these conditions the tumour promoter enhances the probability of the endogenous and unavoidable DNA lesions being expressed as a tumour.

Stimulation of cell division

Stimulation of target cell division for clonal expansion seems to be one common activity of tumour promoters[18]. As carcinogenesis requires the accumulation of a certain number of genetic changes, it is obvious that the rate of fixation of critical lesions in the genome can be accelerated by more rapid cell division. If the hypothesis that some DNA damage is unavoidable is correct, tumour promoters will increase the tumour incidence in a bioassay by promoting spontaneously initiated cells[19].

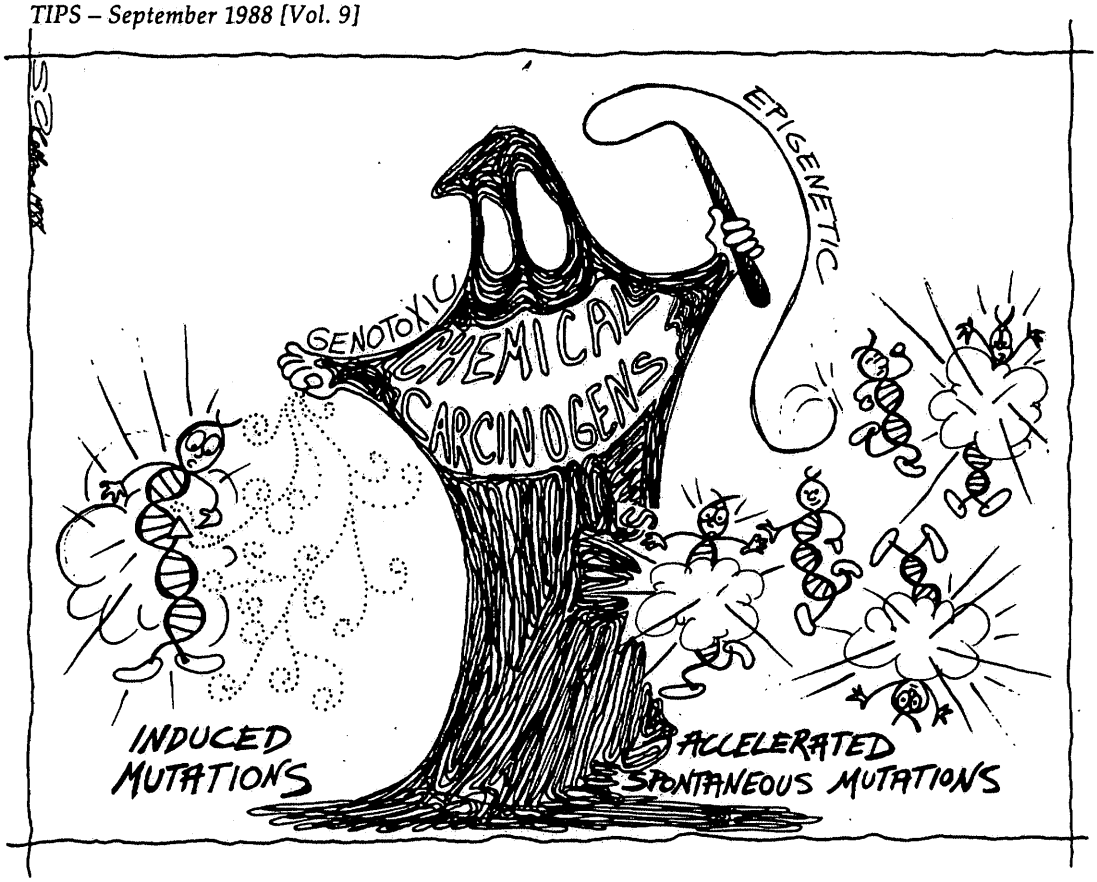
An important distinction might be introduced at this point with respect to the different mechanistic possibilities of inducing a mitogenic response:

- Tumour promoters could act directly as growth factors (estrone, diethylstilbestrol, perhaps TCDD)[20], or interact with already modified growth factor receptors.
- They could stimulate growth factor production and release[21] or affect growth regulators further downstream (e.g. protein kinase C activation).
- They could shift differentiated, resting cells from G_0 to G_1 cell-cycle phase (phenobarbital)[22].
- They could induce restorative growth due to their cytotoxic activity (asbestos fibres, phenacetin, chloroform, genotoxic agents at high dose levels).
- They could inhibit cellular differentiation processes[23] which usually ensure that maturing cells stop dividing.
- They could interupt cell–cell communication, which seems to be necessary for stem cells to divide[24].

Endogenously induced proliferation of initiated cells

If stimulation of cell division is of prime importance for tumour promotion, development and growth of the fertilized egg to the adult organism must be a critical period for tumour promotion. However, uncontrolled proliferation is not compatible with organogenesis. In the adult, controlled stem cell division will be required for maintenance of most tissues. Therefore, the stem cells carrying mutations might be at the highest risk of becoming tumourigenic. Since blood-producing stem cells are particularly active, this may be the reason for a high incidence of leukaemias at a young age.

A transient endogenous stimulation of cell division could induce autonomous growth, not only by efficient fixation of primary DNA lesions, but also in combination with constitutively



controlled genes involved in cell proliferation. Thus other already affected but dormant genes involved in late steps of growth control might be activated. By a defective feedback mechanism[25], autonomous growth could be maintained via autocrine secretion of growth factors. This explains why tumour promoters can achieve their effect by growth stimulation even when given a long time after the exposure to a mutagenic carcinogen.

Cytotoxicity and growth

The proliferative stimulus following tissue damage is a well regulated process, especially in cells that are responsible for repair processes, such as fibroblasts. These cells synthesize and release growth factors which can stimulate growth of tumour cells *in vitro* by a paracrine mechanism[26]. This indirect stimulation might also affect preneoplastic cells and could also function *in vivo*.

Clonal expansion

Another line of evidence suggests that tumour promoters can convey a survival advantage to initiated cells.

One hypothesis is deduced from the study of preneoplastic foci in liver under the influence of tumour promoters such as phenobarbital or halogenated pesticides like DDT or α-hexachlorocyclohexane. The postponed programmed cell death (apoptosis) *in vivo*[27] and the arrest of processes involved in increasing ploidy in hepatocyte cultures[28] caused by these tumour promoters suggest that promotion is due to an altered or arrested differentiation process. Through this, a proliferation of stem cells is maintained with a few cells carrying the genetic alteration necessary to become a tumour cell in the tissue; this interpretation is supported by the observation that among (originally tetraploid) hepatocytes of carcinogen-treated rats, a diploid (stem) cell population preferentially emerges in tumours[29].

A second mechanism was deduced from experiments in which, after treatment of rats with a genotoxic hepatotoxin, cell populations arise in the liver that respond to growth stimuli (partial hepatectomy) but resist the cytostatic/cytotoxic activity of a second hepatocarcinogen[30]. Enzymes able to activate hepatocarcinogens seem to be expressed only at low levels in proliferating cells and therefore the already dividing cells have a further growth advantage. This mechanism might, however, only play a role in specific cases of chronic intoxication.

Genotoxic activity of tumour promoters

Evidence exists that some tumour promoters [e.g. 12-*O*-tetradecanoylphorbol-13-acetate (TPA or PMA)] not only stimulate cell proliferation but also induce chromosome aberrations[31,32]. Furthermore, recombinogenic events have been induced in embryos of pregnant mice by tumour promoters[33], and point mutations are found in cells from epidermal papillomas and carcinomas that occur in mice after treatment with the phorbol esters TPA or mezerein alone[34]. It must therefore be postulated that at least some promotion steps result in genotoxic effects other than binding to DNA and not detectable in presently available gene mutation tests. The uncertainty about the relevance of chromosome aberrations and recombination in the promotion step is mainly caused by the lack of mammalian test systems in which the whole spectrum of mutagenic events can be followed specifically in the few initiated cells of a tissue.

Tumour progression

Since a dividing cell has an advantage over a resting cell with respect to transmitting its genetic information to the progeny, a permanent selection process will favour those cells that have accumulated the largest number of changes towards a malignant (infiltrative and metastatic) cancer cell phenotype. Cell death and regeneration are under close control of hormones, growth factors, intercellular communication and cell surface antigens; any interference with these steps can lead to an increased probability of rapid autonomous tumour growth.

Tumour progression to malignancy is often associated with gross structural and numerical chromosomal abnormalities. However, it is believed that this massive destabilization of the genome without clonal origin is not the prerequisite for efficient cell proliferation.

Need for mechanistic distinction

The distinction between genotoxic and epigenetic activity of carcinogens (Fig. 1) might seem rather theoretical for the cancer victim. However, we consider it essential to understand the biological processes underlying the process of carcinogenesis, since it is an important aspect when data obtained from animals are to be extrapolated to humans. For example, when carcinogens are found to be transformed into chemically reactive metabolic intermediates interacting with DNA in some animal species, it will be important to find out whether the compound is metabolized in a similar way in humans. Additionally, the dose–response relationship might be different for genotoxic carcinogens and those carcinogens that act only at high dose levels by inducing cytotoxicity and regenerating cell division, or those non-mutagenic carcinogens that act at hormonal levels[35].

An understanding of the mechanisms of carcinogenic activity might allow for the development of a battery of short-term tests able to detect chemicals active in defined steps of carcinogenesis. This will facilitate better risk evaluation of chemical carcinogens. New tests to be devised should permit a comparative analysis of different types of mutations and the detection of chemicals that stimulate cell proliferation and interfere with cell differentiation.

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TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin
DDT: 1,1,1-trichloro-2,2-bis(*p*-chloro-phenyl)ethane

Pharmacological approaches to the modulation of plasma cholesterol

James Shepherd and Christopher J. Packard

Several recent clinical trials have demonstrated that strategies that lower plasma cholesterol levels do indeed have a beneficial effect on events related to coronary heart disease. A major strategy to lower cholesterol levels is via pharmacological intervention. James Shepherd and Christopher Packard *evaluate the mechanisms and effectiveness of currently available classes of drug that act by lowering sterol absorption, lowering sterol synthesis, or interrupting the enterohepatic circulation.*

Health care services in industrialized countries are becoming so bogged down in the treatment of established disease, that little is left to offer the population by way of preventive therapy. As a consequence, chronic degenerative illnesses are placing an ever increasing burden on the resources of primary health care systems, despite accumulating evidence that some of them may be improved by simple measures aimed at lifestyle modification.

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Coronary artery disease, for example, which is responsible for about a third of all deaths in industrial societies, appears to respond to interventions designed to alter population attitudes and habits. Several major risk factors – hypercholesterolaemia, hypertension and cigarette smoking – contribute to its development, and when treated produce significant advantage for the individual. The Lipid Research Clinics Coronary Primary Prevention Trial[1], for example, offered convincing evidence that lowering plasma cholesterol levels in asymptomatic hypercholesterolaemic men reduces their risk of having a myocardial