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FACULTY OF ENGINEERING & TECHNOLOGY

Chemical Carcinogenesis & Metabolism

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Chemical carcinogenesis

•Chemicals appear to be of major importance in the induction of human cancers. The known chemical carcinogens include a wide range of structures. Their common feature is that their ultimate forms are electrophilic reactants; in most cases, these reactants arise through metabolism in vivo.

•Carcinogenesis by chemicals is a multistage process.

Cancer arise in 3-steps

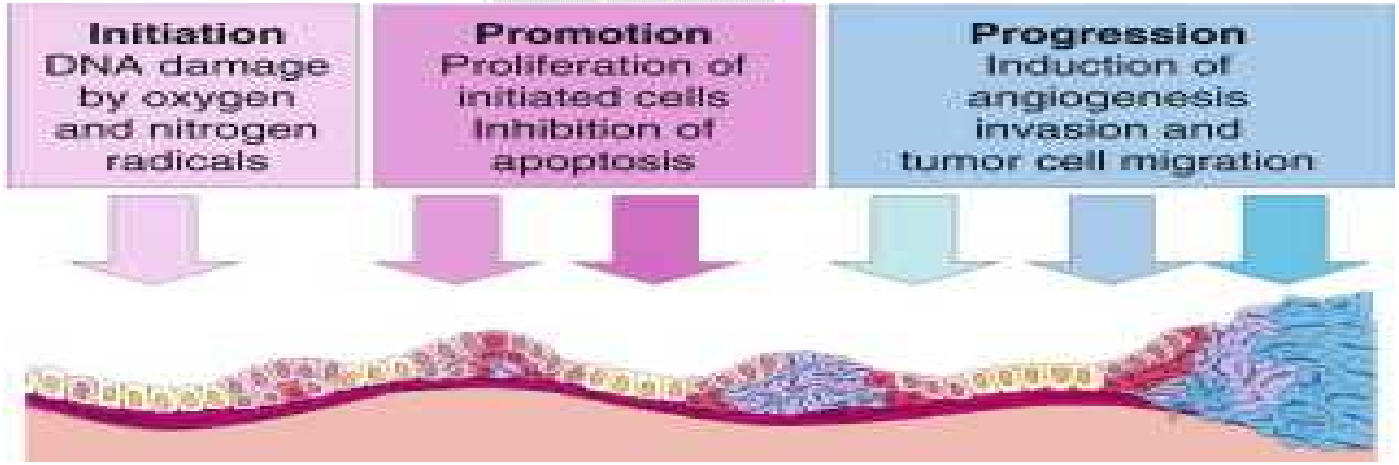
i. Initiation (the process of acquisition of genetic and epigenetic changes that sets the cell on path to cancer),

ii. Promotion (a step during which the primed cells express altered responses that provide a selective advantage allowing them to survive and develop locally),

iii. Progression (a series of steps during which the now established cancer cells accumulate further changes on the path to malignancy

•Chronic inflammation contributes to each of these mechanisms. First, inflammation generates an overload in reactive oxygen species (ROS) and reactive nitrogen species (RNS) that damage DNA and enhance several mutagenic processes, thereby accelerating the acquisition of mutations that drive the cancer process. Second, chronic inflammation involves the production of a complex combination of factors, some of which promote cell proliferation and survival, while others induce cell death.

•Third, inflammation profoundly alters the relationships between cells and their stroma, and also enhances angiogenesis, thus facilitating local invasion and distal dissemination of cancer cells.



•The role of inflammation in initiation, promotion, and progression stages of cancer development. The sequence of development of an epithelial tumor is represented and divided in three steps: initiation, promotion, and progression. The contribution of inflammatory mechanisms to each step is summarized.

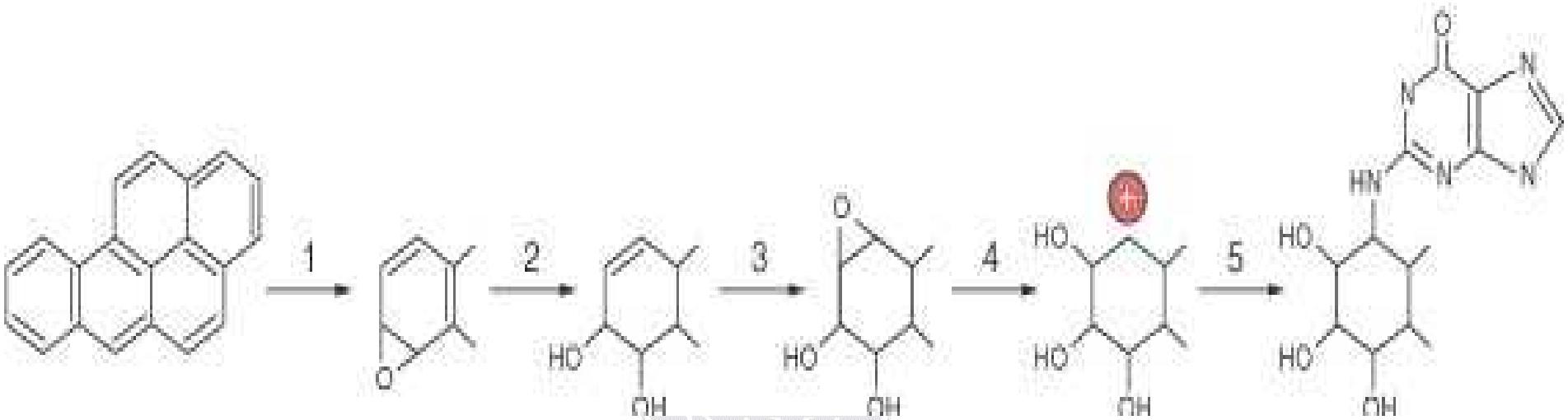
Different type of electrophilic reactant generated *invivo*.

The main DNA-damaging reactive species produced during inflammation are hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\cdot}$), and reactive intermediates such as hydroxyl radicals ($OH^{\cdot}$), superoxide ($O_2^{-\cdot}$), and peroxynitrite ($ONOO^-$).During inflammatory response, $NO^{\cdot}$ is also produced inside cells through the activation of the transcription of inducible nitric oxide synthase (NOS)-2 in response to cytokines. The promoter of NOS-2 contains binding sites for nuclear factor kappa B (NFκB). Overproduction of $NO^{\cdot}$ causes two main mechanisms of mutagenesis. One is direct DNA damage through radical attack of DNA (generating DNA strand break, base damage, and chromosome damage). The second is enhanced deamination of 5-methylcytosine (5mC), the most common methylated form of cytosine representing about 3% of all cytosines in the genome. Deamination of 5mC into thymine generates a DNA mismatch (G:T) whichif not repaired, may result in a mutation (from a G:C base pair to an A:T base pair) Since 5mC preferentially occurs at CpG dinucleotides, this type of mutation is often found within this particular sequence context. Mutations at CpG dinucleotides are the most frequent form of single base substitutions in inflamed tissues and in cancers arising in an inflammatory context. For example, about 50% of mutations in the TP53 tumor suppressor gene occur at CpG dinucleotides in colon cancer and in adenocarcinoma of the esophagus – two cancers with well-defined inflammatory precursors.

Metabolism of chemical carcinogenesis

•The first chemically identified carcinogens were the polycyclic aromatic hydrocarbons (PAH).They are composed of variable numbers of fused benzene rings that form from incomplete combustion of fossil fuels and vegetable matter (including tobacco), and they are common environmental contaminants. The polycyclic aromatic hydrocarbons are chemically inert, and require metabolism to exert their biologic effects.

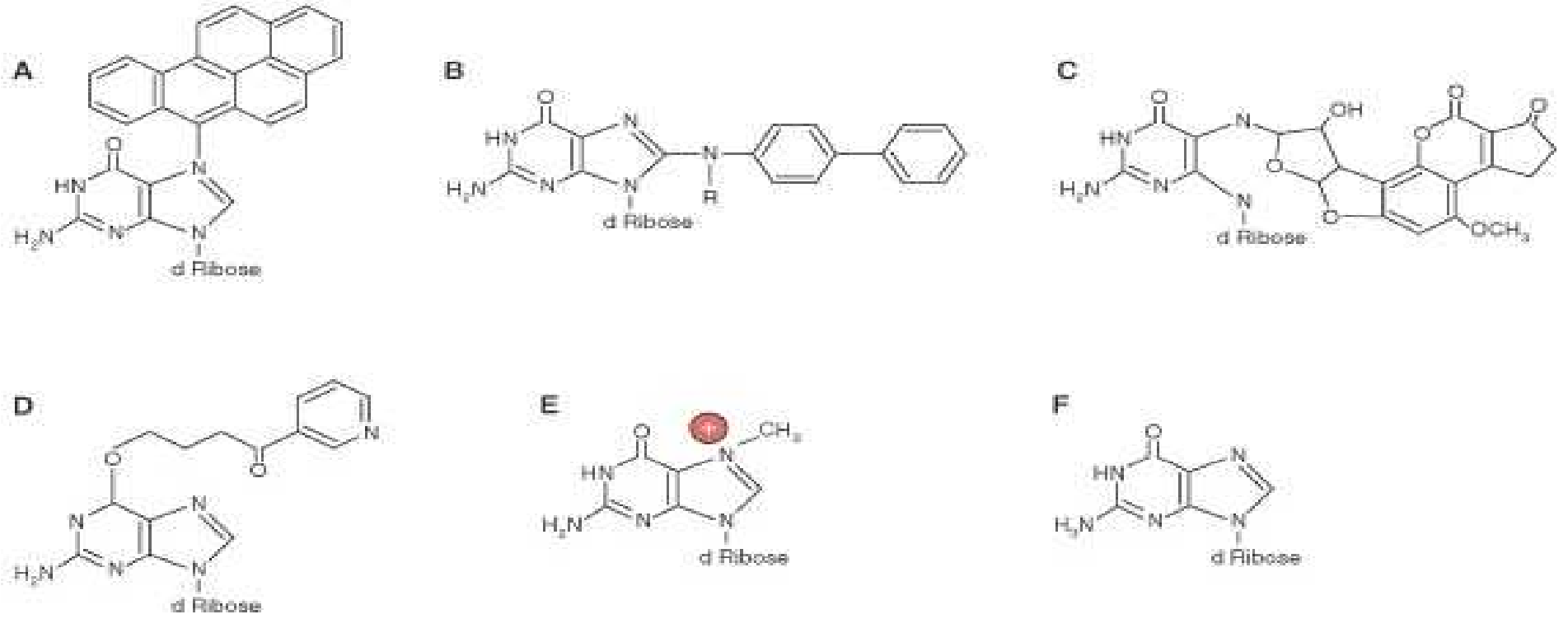
•This is a multistep process, it involves the following: initial epoxidation (cytochrome P450, CYP1A1 is an inducible isoform), hydration of the epoxide (epoxide hydrolase), and subsequent epoxidation across the olefinic bond (CYP1B1; CYP3A4). The result is the ultimate carcinogenic metabolite, a diolepoxide



Metabolic activation of benzol[α]pyrene. (1) Cytochrome P450 (CYP1A1) catalyses initial epoxidation across the 1 - 2, 2 - 3, 4 - 5, 7 - 8 (shown), 9 - 10 and 11 - 12 positions. (2) With the exception of the 1 - 2 and 2 - 3 oxides that convert to phenols, epoxide hydrolase may catalyze the formation of dihydrodiols. (3) Benzo[*a*]pyrene-7, 8-dihydrodiol is further metabolized at the olefinic double bond by cytochrome P450 (CYP1B1 and CYP3A4) to form a vicinal diol-epoxide (r7, t8-dihydroxy-c9, 10 epoxy-7,8,9,10-tetrahydroxybenzo[*a*]pyrene). (4) The highly unstable arene ring opens spontaneously to form a carbocation. (5) This electrophic species forms a covalent bond between the 10 position of the hydrocarbon and the exocyclic amino group of deoxyguanosine.

•The arene ring of benzo[*a*]pyrene-7,8-diol 9,10-oxide opens spontaneously at the 10 position, giving a highly reactive carbonium ion that can form a covalent addition product (ie, adduct) with cellular macromolecules, including DNA. An alternative pathway of PAH activation, through a mechanism of one electron oxidation, has also been postulated.

•When benzo[*a*]pyrene is activated by this route, the resulting radical cation is formed at the *meso* position or L-region.The reactive cation forms DNA adducts at the C8 of guanine (BP-6-C8Gua and BP-6-C8dGua), the N7 of guanine (BP-6-N7Gua), and the N7 of adenine (BP-6-N7ade) . These adducts place strain on the *N*-glycosyl link to the helical backbone and depurination results. Firm evidence for the exfoliation of these adducts in urine was provided recently for exposure scenarios that included coal and tobacco smoke.



Examples of carcinogen-DNA adducts: **A,** N7(benzo[α]pyren-6-yl)guanine; **B,** *N*-(deoxyguanosin-8-yl)-{acetyl}aminobiphenyl (when R= H the adduct is not acetylated [R can also be an acetyl group]); **C,** 8,9-dihydro-8-(N^5-formyl- 2′, 5′, 6′-triamino-4′-oxo-N^5-pyrimidyl)-9-hydroxy-aflatoxin B_1; **D,** O^6-[4-Oxo-4(3-pyridyl)butyl]guanine, a mutagenic lesion formed by the metabolism of the tobacco-specific nitrosamine, NNK; (E) N7-methyldeoxyguanosine; and (F) 3-methyladenosine. Adducts **E,** and **F,** can also result as the small alkyl products of NNK metabolism.

An initial activation step for both aromatic amines and amides is *N*-oxidation by CYP1A2. *CYP1A2* is inducible by phenobarbital, and because it is also responsible for the 3-demethylation of 1,3,7-trimethylxanthine (ie, caffeine), *CYP1A2* phenotype can be determined using this as a probe drug. The reactions of *N*-hydroxy-arylamines with DNA appear to be acid catalyzed, but they can be further activated by either an acetyl coenzyme A-dependent O-acetylase or a 3′-phosphoadenosine-5′phosphosulfate-dependent O-sulfotransferase. The *N*-arylhydroxamic acids arise from the acetylation of *N*-hydroxy-arylamines or *N*-hydroxylation of aromatic amides; they are not electrophilic and require further activation. The predominant pathway for this occurs through the acetyltransferase-catalyzed rearrangement to a reactive *N*-acetoxy-arylamine. Sulfotransferase catalysis forms *N*-sulphonyloxy arylamides. This complex pathway results in two major adduct types, amides (ie, acetylated) and amines (ie, nonacetylated).

The heterocyclic amines form while cooking food, primarily from the pyrolysis (>150°C [302°F]) of amino acids, creatinine, and glucose. They have been recognized as food mutagens, shown to form DNA adducts and cause liver tumors in primates.These compounds are activated by CYP1A2, and their metabolites form DNA adducts in humans.The *N*-hydroxy metabolites of 3-amino-1-methyl-5*H*- pyrido[4,3-b]indole (Trp-P-1), 2-amino-6-methyldipyrido[1,2-a:39,29-d]imidazole (Glu-P-1), and 2-amino-3-methyl-imidazo- [4,5-f]quinoline (IQ) can react directly with DNA. Enzymic O-esterification of *N*-hydroxy metabolites plays a key role in activating food mutagens, and the *N*-hydroxy metabolites are also good substrates for transacetylases. This suggests a possible etiologic role for these chemicals in colorectal cancer with the rapid acetylator phenotype.

Aflatoxins (B_1, B_2, G_1, and G_2) are metabolites of *Aspergillus flavus* that contaminate cereals, grain, and nuts. A positive correlation exists between dietary aflatoxin exposure and the incidence of liver cancer in developing countries, where grain spoilage is high. Aflatoxins are activated by several cytochrome P450s (CYP2A3; CYP2A6; CYP3A4). Aflatoxin B_1 and G_1 have an olefinic double bond at the 8,9-position, and they are more mutagenic and carcinogenic than aflatoxin B_2 and G_2, which are saturated and have an ethylenic bond at this position. This implies that the olefinic 8,9-bond is the activation site. Further support for this mechanism comes from studies of DNA-adducts and the prevalence of p53 mutations in liver cancer. In people with liver cancer from parts of China and Africa, where food spoilage caused by molds is high, G:C to T:A transversions in codon 249 are frequent. This phenomenon is consistent with metabolic activation of aflatoxin B_1 and the formation of depurinating carcinogen-deoxyguanosine adducts.

Carcinogenic *N*-nitrosamines are ubiquitous environmental contaminants and can be found in food, alcoholic beverages, cosmetics, cutting oils, hydraulic fluid, rubber, and tobacco. Tobacco-specific *N*-nitrosamines, such as 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone, are carcinogenic in a wide range of animal species. Unlike exposure to many other carcinogens associated with tobacco use, exposure to tobacco-specific *N*-nitrosamines does not require pyrolysis, therefore they may account for the carcinogenic nature of snuff and chewing tobacco. The tobacco-specific nitrosamines are not symmetric so both small alky-adducts and large bulky adducts can be formed; for example, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone metabolism gives rise to either a positively charged pyridyl-oxobutyl ion or a positively charged methyl ion, both of which are able to alkylate DNA.

Endogenous nitrosation forms nitrosamines when an amine reacts with nitrate alone or nitrite in the presence of acid. Thus, nitrite (used in curing meats) and l-cysteine, in the presence of acetaldehyde (a metabolite of alcohol), form *N*-nitrosothiazolidine-4-carboxylic acid. *N*-nitrosodimethylamine undergoes α-hydroxylation, catalyzed primarily by the alcohol inducible *CYP2E1*, to form an unstable α-hydroxynitrosamine. The breakdown products are formaldehyde and methyl diazohydroxide. Methyl diazohydroxide and related compounds are powerful alkylating agents that can add a small functional group at more than 10 different DNA sites.

References & Further reading

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