Hepatocarcinogenesis: chemical models

Introduction

- Earliest observations that human exposure to certain chemicals is related to an increased incidence of cancer
- John Hill 1761
  - Nasal cancer in snuff users
- Sir Percival Pott 1775
  - Scrotal cancer in chimney sweeps
  - Soot and coal tar

Experimental chemical carcinogenesis

- Yamagiwa and Ichikawa 1918
- Multiple applications of coal tar to rabbit ears produced skin carcinomas
- First demonstration that a chemical could produce cancer in an animal
- Confirmed Pott's initial observation and linked human epidemiology and animal carcinogenicity

Somatic mutation theory

- Theodor Boveri 1914
- Concept that cancer involves an alteration in the genetic material of the somatic cell
  - Chromosome abnormalities
- Furth and Kahn 1934
- Isolated single cell clones from a tumor and showed that injection into a healthy host could reproduce disease
  - Cancer = stable heritable cellular alteration

Chemical carcinogenesis

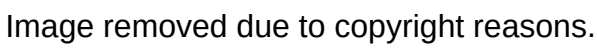
- James and Elizabeth Miller 1950s
- Observed that a wide variety of structurally diverse chemicals could produce cancer in animals
- Proposed that all of these chemicals require metabolic activation to electrophilic reactive intermediates
  - Covalently bind to nucleophilic centers on proteins, RNA, or DNA
  - Electrophilic theory of chemical carcinogenesis

Evidence for genetic mechanisms

1) Cancer is a heritable stable change
2) Tumors are generally clonal in nature
3) Many carcinogens are metabolized to electrophilic intermediates that covalently bind to DNA
4) Many carcinogens are also mutagens
5) Many cancers display chromosomal abnormalities
6) Transformed phenotype can be transferred from a tumor cell to a non-tumor cell by DNA transfection

Genotoxic agents

- Direct acting carcinogens
  - *N*-methyl-*N*-nitrosourea (MNU)
  - *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)
- Indirect acting carcinogens
  - Dimethylnitrosamine (DMN)
  - Benzo[a]pyrene
- Radiation
- Inorganic agents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Epigenetic agents

- Immunosuppresive xenobiotics
- Asbestos
- Hormones
- Promoters
  - 12-O-tetradecanoylphorbol-13-acetate
  - Phenobarbital

Evidence for epigenetic mechanisms

1) Cancer is associated with altered differentiation and proliferation
2) The cancerous state of tumors is sometimes reversible
3) Carcinogenesis is induced by non-mutagenic substances
4) Not all carcinogens are mutagens
5) Carcinogenesis is associated with changes in DNA methylation

Multistage carcinogenesis

- Initiation
  - Genotoxic event
- Promotion
  - Clonal expansion of an initiated cell
- Progression
  - Development of a malignant tumor

Initiation-promotion model

- 12-O-tetradecanoylphorbol-13-acetate (TPA) belongs to a family of compounds called phorbol esters that are isolated from croton oil and are active almost exclusively on mouse skin
- TPA is also known as phorbol 12-myristate 13-acetate (PMA)
- Phenobarbital, DDT, chlordane and TCDD are hepatic tumor promoters

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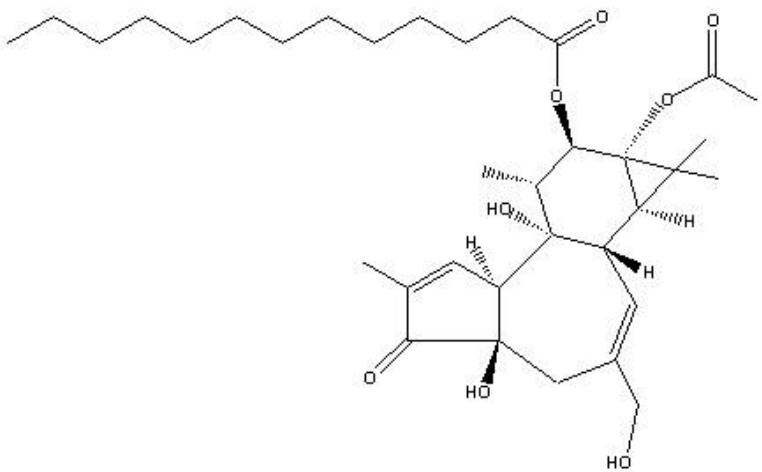


Features of tumor promoters

1) Following a sub-threshold dose of an initiating carcinogen, chronic treatment with a tumor promoter will produce many tumors
2) Initiation at a sub-threshold does alone will produce very few if any tumors
3) Chronic treatment with a tumor promoter in the absence of initiation will produce very few if any tumors
4) The order of treatment is critical: initiation must precede promotion

Mouse skin model

- Berenblum 1941
  - Alternating doses of croton oil and benzo[a]pyrene
- Mottram et al.
  - Single sub-effective dose of benzo[a]pyrene followed by repetitive croton oil treatments

1) SW mice 200 nmol DMBA
2) 1 week later, 2-5 nmol TPA twice a week for 20 weeks
3) After 15 weeks, 12-14 benign papillomas

Mechanisms of tumor promotion

- Clonal expansion of initiated cells by providing a selective growth advantage, or by repressing normal cell growth, or both
- The specific phorbol ester is protein kinase C (PKC)
  - Serine and threonine kinase and a Ca^{2+} and phospholipid-dependent enzyme
  - Diacylglycerol is also a potent tumor promoter in mouse skin

Rodent models of liver cancer

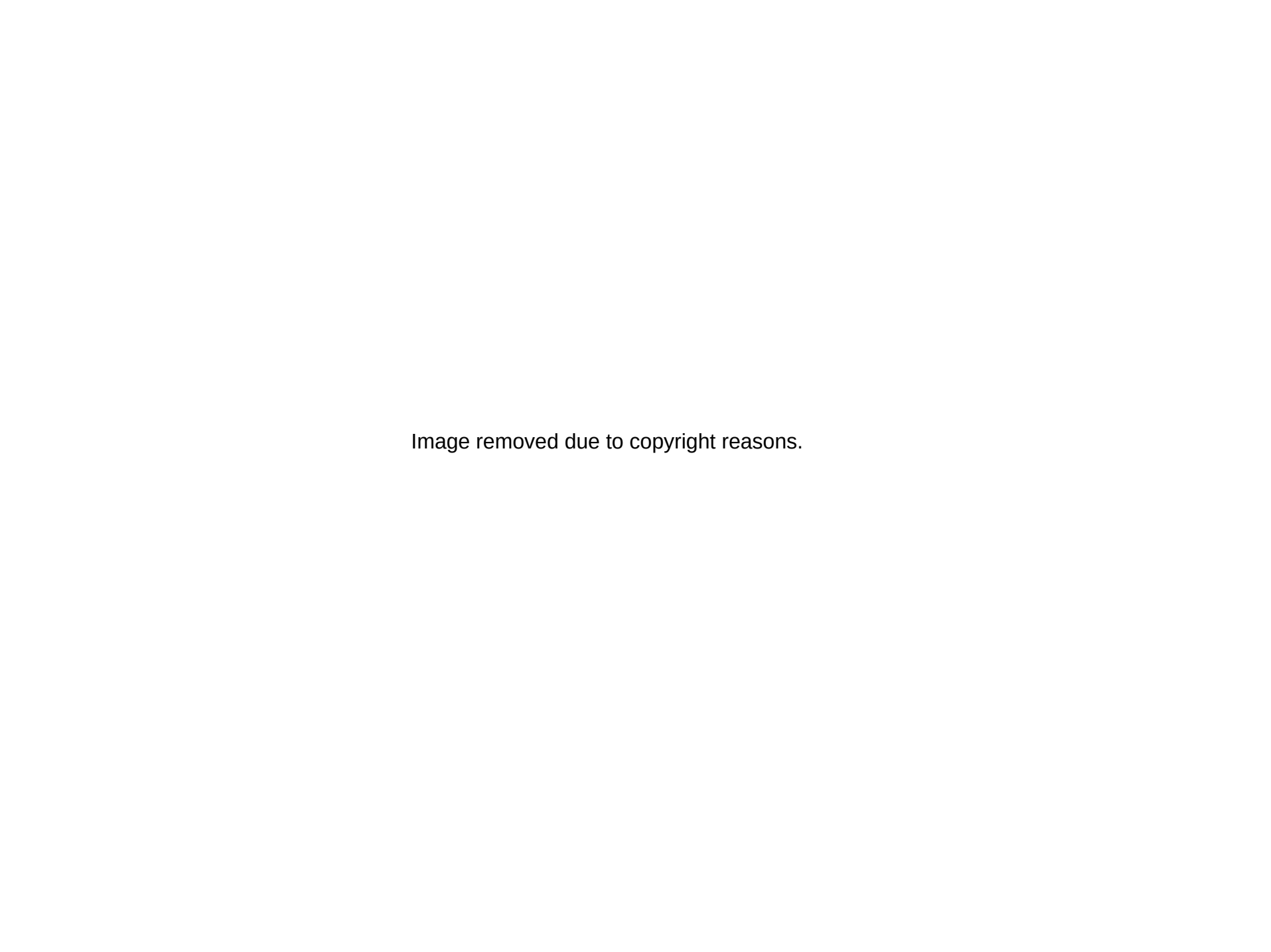
- Most rat strains have < 5% lifetime incidence of primary hepatocellular tumors
- In contrast, outbred Swiss Webster mice have 35% incidence in males and 5% incidence in females
- In the B6C3F1 (National Toxicology Program; NTP) mouse the range is 25-40% for males and 4.6-9.7% for females
- In bioassays for carcinogenicity, the liver is the most commonly affected site

Hepatic carcinogenesis

- 2 major pathways have been described
  - Oval cell proliferation leading to lesions composed of extensive connective tissue matrix investing a metaplastic ductal system (cholangiofibrosis or adenofibrosis)
  - Altered hepatic foci, hepatic nodules, and hepatocellular carcinoma (HCC)
- Much of our current understanding comes from nitrosamine or aflatoxin studies in rats (relatively non-toxic at carcinogenic doses)

Altered hepatic foci

- Hepatocellular tumors develop from foci of altered hepatocytes
- Increased eosinophilia, or basophilia, or because of rearrangement of RER, may be striped or tigroid in appearance
- In the rat, many foci express fetal enzymes such as gamma-glutamyl transferase (GGT) and the placental form of GSH S-transferase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Some aspects poorly understood

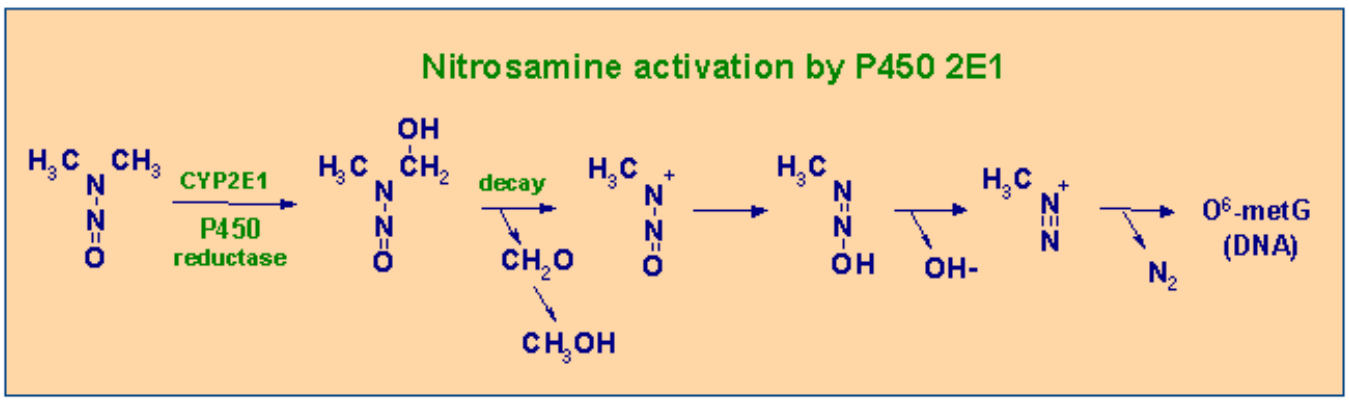
- The changes are not seen in all foci
- Foci in mice do not have GGT or placental GSH S-transferase
- Whether all foci develop into tumors is not known
- The origin of the foci is also not known
- As they grow, the foci become nodules

2-step hepatocarcinogenesis

- Initiation followed by promotion
- Rodents appear to have no absolute requirement for deliberate exposure to genotoxic carcinogens for neoplasia to develop
  - Spontaneously initiated cells in the liver
  - Low-level environmental exposure to genotoxic carcinogens or inherent metabolic processes leading to oxidative stress?

Genotoxic hepatocarcinogens

- Metabolic activation of dimethylnitrosamine (DMN) or diethylnitrosamine (DEN)
- Ultimate carcinogen is methyl diazonium ion
- Methyl carbonium ion forms pre-mutagenic O^6-guanine and O^4-thymidine



Epigenetic hepatocarcinogens

- 2 classes have been widely investigated
  - Phenobarbital (PB)
  - Peroxisome proliferators
- PB causes induction of mixed function oxidase enzymes
- Causes liver enlargement as well as CYP enzyme induction
  - Hyperplasia, hypertrophy of cells in centrilobular region (due to proliferation of SER)

PB promotion

- If PB is given to rats for ≥ 18 months, there may be a small increase in the number of hepatic tumors
- If treatment is preceded by short exposure to genotoxic carcinogen such as DEN, administration of PB results in considerable tumor burden
- With PB treatment, foci have up to 10-fold increase in mitotic activity and decreased apoptosis

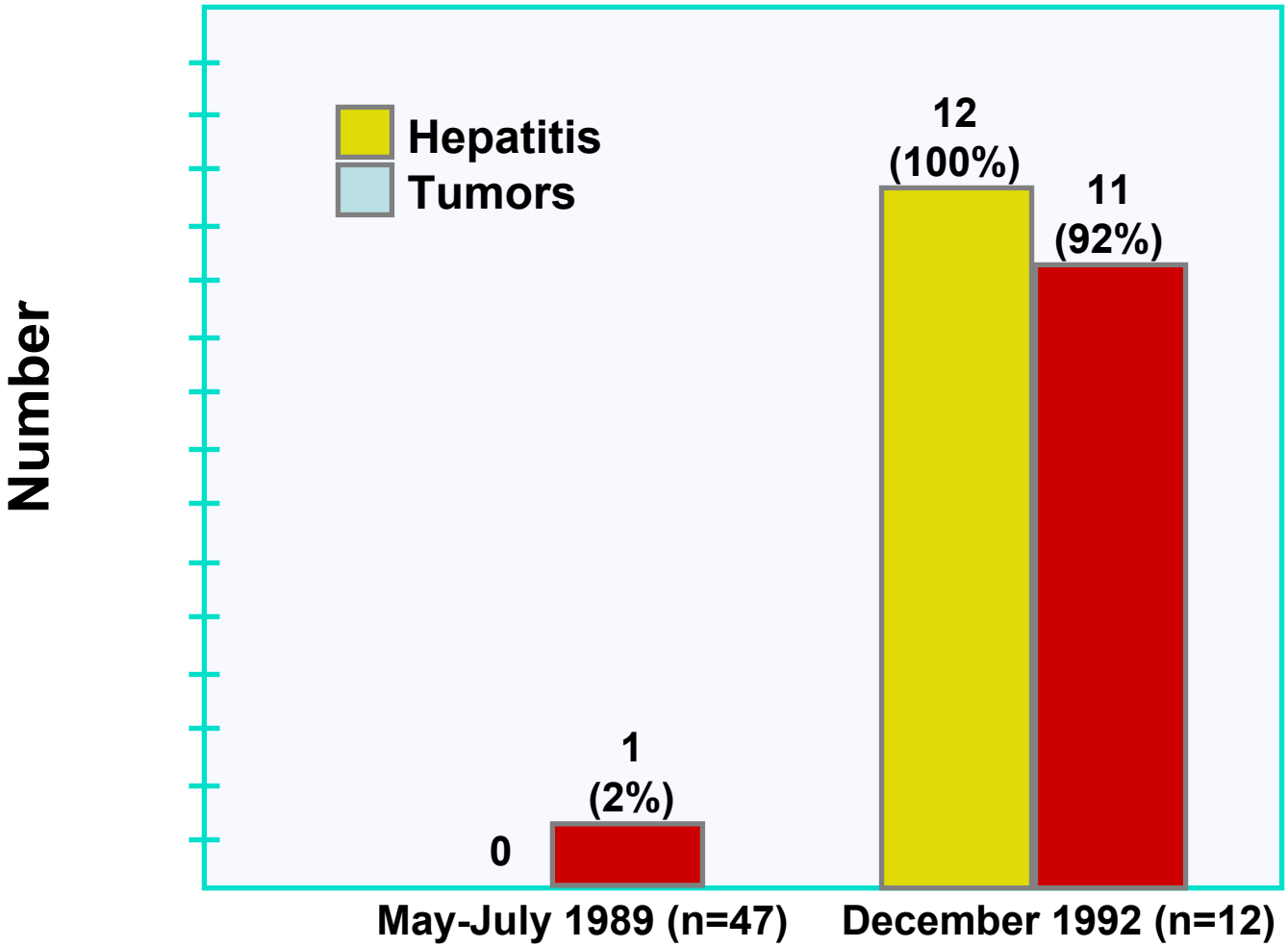
Peroxisome proliferators

- Chemically heterogeneous group
- Phthalate esters most widely studied
  - Hypolipidemic agents based on clofibric acid, or unrelated tibric acid, and WY-14643
- Mice and rats > hamsters > guinea pig > primates
- Hyperplasia and cellular hypertrophy with massive expansion in size and number of peroxisomes (approximately 10-fold increase)
- Cytoplasmic receptors belong to steroid hormone receptor superfamily are peroxisome proliferator activated receptors (PPARs)

Peroxisome induced tumors

- Chronic administration of agents that induce peroxisome proliferation results in accumulation of lipofuscin in the liver and development of HCC in mice and rats
- Basophilic foci give rise to basophilic nodules, then to trabecular carcinomas
- Different from spontaneous foci in the rat
  - Negative for GGT and placental GSH S-transferase
- Hyperplasia plus oxidative stress

Helicobacter-Associated Hepatitis and Hepatocellular Neoplasms in Control A/JCr Male Mice



Fox *et al* 1994
Ward *et al* 1994

H. hepaticus in A/J mouse liver and colon

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Similar Paradigm for *Helicobacter hepaticus* Progression of Pre-Malignant Liver Changes

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Lobular Hepatitis **Dysplasia** **Hepatocellular carcinoma**