The Use of Short- and Medium-term Tests for Carcinogens and
Data on Genetic Effects in Carcinogenic Hazard Evaluation
D.B. McGregor, J.M. Rice and S. Venitt, eds
IARC Scientific Publications No. 146
International Agency for Research on Cancer, Lyon, 1999

Chemically Induced Preneoplastic Lesions in Rodents as Indicators of Carcinogenic Activity

G.M. Williams

Introduction

One of the earliest reports of chemically induced neoplasia — liver cancer induced in rats by *ortho*-aminoazotoluene by Sasaki and Yoshida (1935) — described foci of abnormal hepatocytes which preceded the occurrence of liver tumours. Subsequently, similar observations of preneoplastic lesions were made in other tissues, notably skin and mammary gland. In 1969, Foulds, in a classic treatise, compiled extensive evidence that the evolution of cancer is a multistep process involving antecedent preneoplastic lesions. Later reviews extended information on the pathogenesis of chemically induced cancer in experimental animals (Berenblum, 1974; Farber & Cameron, 1980).

On the basis of the concept that preneoplastic lesions are predictive of later cancer development, investigators have used preneoplastic or early neoplastic lesions in various tissues as end-points in studying the carcinogenic activity of chemicals (Weisburger & Williams, 1984; Bannasch, 1986a,b; Montesano *et al.*, 1986). In order that a lesion be a valid indicator of carcinogenic activity, it must be shown to be composed of an abnormal population of cells that are precursors of neoplasms. This review summarizes the evidence for the reliability of induction of preneoplastic lesions in various organs in rodents as indicators of carcinogenic activity. The order in which the organs are presented roughly follows the extent of evidence for (i) the availability and reliability of preneoplastic lesions as surrogates for tumours and (ii) the application of those surrogates.

A number of biochemical and biological properties can be considered to provide evidence for the preneoplastic nature of lesions. The availability of data on such lesions for each organ is summarized in Table 1.

Preneoplastic lesions in the liver

Study of experimental liver carcinogenesis began with the report by Sasaki and Yoshida (1935) of the induction of hepatocellular cancer in rats by *ortho*-aminoazotoluene. Subsequent research extended their observation that the evolution of chemically induced hepatocellular cancer in rodents is a multistep process which proceeds through distinctive stages (Firminger, 1955; Daoust & Molnar, 1964; Reuber, 1965; Bannasch, 1968; Kitagawa, 1971; Farber, 1973; Williams, 1976; Pitot *et al.*, 1978). Other liver neoplasms, such as cholangiocarcinomas and hepatoblastomas, are produced by chemicals, but their pathogenesis have not been as thoroughly studied.

Hepatocellular altered foci (HAF), or hyperplastic foci, are lesions that occur early in hepatocarcinogenesis (Firminger, 1955; Reuber, 1965; Bannasch, 1968; Daoust & Calamai, 1971; Kitigawa, 1971) and are generally accepted to be a progenitor of subsequent lesions (Bannasch, 1986a; Williams, 1989; Pitot, 1990), including the more advanced hyperplastic nodule (Kitigawa, 1971; Farber, 1973; Becker, 1976). HAF are composed of hepatocyte-like cells of inconstant size, morphology and cytoplasmic, nuclear and nucleolar staining reactions; nucleoli are often prominent. The composition of the cells in each focus is fairly uniform, and they have clear, eosinophilic or basophilic (tigroid) cytoplasm (Stewart *et al.*, 1980; Bannasch & Zerban, 1990). The cells of HAF are arranged in plates that are little altered from normal and merge with those of the surrounding parenchyma without compression. An indication of their abnormal

Table 1. Evidence for the preneoplastic nature of chemically induced lesions

Evidence	Liver (HAF)	Skin (EH/EP)	Lung (AD)	Mammary gland (HAN)	Colon (ACF)	Kidney (ATH)	Pancreas (AACF)
Similarity of morphological and biochemical changes to those in tumours	+	+	+	+	+	+	+
Genetic alterations	+	NR	+	NR	+	NR	+
Appearance before experimentally induced tumours	+	+	+	+	+	+	+
Persistence after cessation of exposure to carcinogen	+/–[a]	+/–[a]	+	+	+	+	+
Transition into neoplasms	+	+	+	+	NR	+	+
Transplantability	-	+	+	+	NR	NR	NR
Correlation with tumours	+	+	+	+	+/–	+	+
Response to promoters	+	+	+	+	+	NR	NR

HAF, hepatocellular altered focus; EH, epidermal hyperplasia; EP, epithelial papilloma; AD, adenoma; HAN, hyperplastic alveolar nodule; ACF, aberrant crypt focus; ATH, atypical tubule hyplasia; AACF, atypical acinar-cell focus; NR, not reported

[a] Some undergo regression.

cellular function is a deficit of fibronectin (Maruyama *et al.*, 1988). Hyperplastic nodules or adenomas are larger lesions composed of similar cells. In contrast to HAF, they compress the surrounding parenchyma (Stewart *et al.*, 1980; Bannasch & Zerban, 1990).

The cellular origin of HAF may differ with specific inducing agents, but there is persuasive evidence that the precursor cell of well-studied DNA-reactive hepatocarcinogens is the hepatocyte (Karasaki, 1969; Timme, 1978; Hirota & Williams, 1982; Gebhardt *et al.*, 1989), although under some circumstances, such as highly toxic levels of exposure, HAF may originate from stem cells (Sell & Leffert, 1982; Fausto *et al.*, 1992; Thorgeirsson & Evarts, 1992; Marceau *et al.*, 1992). Two agents, 2-acetylaminofluorene and *N*-nitrosodiethylamine, mainly affect a subpopulation of hepatocytes in rats in the centrilobular region that expresses glutamine synthetase, and consequently induce HAF with this phenotype (Gebhardt & Williams, 1995).

HAF cells express a number of phenotypic abnormalities that indicate that they are preneoplastic. These include decreased glucose 6-phosphatase activity (Gössner & Friedrich-Freska, 1964), excess glycogen storage (Bannasch, 1968), decreased ATPase activity (Schauer & Kunze, 1968), reduced β-glucuronidase activity (Kitigawa, 1971), re-expression of γ-glutamyltransferase activity (Kalengayi *et al.*, 1975), reduced iron storage (Williams & Yamamoto, 1972), increased glucose 6-phosphate dehydrogenase activity (Hacker *et al.*, 1982) and re-expression of placental-type glutathione *S*-transferase (Sato *et al.*, 1984). These phenotypic abnormalities are prominent in lesions in rat liver but less so in the livers of other species, although deficiency in iron storage characterizes HAF in all species investigated, including humans (Mori *et al.*, 1989). Individual HAF can express one or more phenotypic abnormalities (Pitot *et al.*, 1978; Hirota & Williams, 1979; Yamaguchi *et al.*, 1993).

The variety of phenotypic abnormalities in HAF probably reflects different alterations in the expression of various genes, which could be due to genetic abnormalities. HAF in rats have been reported to show alterations in nuclear DNA (Mori *et al.*, 1982; Sudilovsky & Hei, 1991), although 75–80% of γ-glutamyltransferase-positive cells isolated from rat livers with HAF were found to be diploid (Sargent *et al.*, 1989).

HAF are proliferative lesions (Karasaki, 1969; Newberne, 1976; Scherer & Emmelot, 1976; Rabes, 1989) in which the degree of cell replication is a function of the phenotype of the constituent cells (Stenbäck *et al.*, 1994; Zerban *et al.*, 1994). The basis for the proliferation and clonal expansion of HAF may vary but obviously reflects a greater rate of cell replication than of cell loss or apoptosis, which counterbalances proliferation (Schulte-Hermann *et al.*, 1995). HAF display reduced gap-junctional intercellular communication (Krutovskikh *et al.*, 1991), which could result in diminished cell growth control. Some HAF also express alterations in the expression of growth control genes (Galand *et al.*, 1988; Kaufman *et al.*, 1992; Bauer-Hofmann *et al.*, 1992; Steinmetz & Klaunig, 1996), and they may be resistant to factors that lead to programmed cell death, thereby increasing their growth rate. A locus for hepatocarcinogen sensitivity, which operates in the promotion stage of tumour induction to increase the growth of preneoplastic lesions, has been identified in mice (Drinkwater *et al.*, 1990). A growth stimulatory effect of androgens is exerted on preneoplastic and neoplastic liver lesions (Rice, 1973; Matsuura *et al.*, 1994). HAF are resistant to the intense toxicity of administered carcinogens (Farber *et al.*, 1976; Williams *et al.*, 1976) because of down-regulation of xenobiotic activation enzyme systems (Cameron *et al.*, 1976; Okita *et al.*, 1976) and because of this can selectively proliferate in an environment that is toxic to normal hepatocytes.

In the absence of exposure to carcinogens or other stimulation, most HAF do not progress or, on the contrary, even undergo regression or phenotypic reversion (Bannasch, 1968; Williams & Watanabe, 1978). In view of their limited growth potential, HAF do not grow progressively after transplantation (Mori *et al.*, 1983a).

A small fraction of HAF progress to form hyperplastic nodules or neoplasms over time or with continued exposure to carcinogens (Reuber, 1975; Williams, 1980; Pitot, 1990); these neoplastic lesions have many of the same phenotypic abnormalities as HAF (Williams, 1980; Mori

et al., 1989). Carcinomas can arise within hyperplastic nodules or benign adenomas, but they can also evolve directly from HAF. In fact, some HAF, particularly in the mouse, have been considered to be microneoplasms (Goldfarb & Pugh, 1992). The role of HAF as precursors of neoplasms was firmly established with the discovery that they respond to promoters of liver neoplasia (Kitigawa & Sugano, 1978; Pitot *et al.*, 1978; Watanabe & Williams, 1978).

In rodents exposed to carcinogens, HAF may precede liver tumours by months or years and outnumber the tumours by 100- or 1000-fold (Watanabe & Williams, 1978; Pitot *et al.*, 1985).

HAF occur spontaneously, especially in species with high background levels of cryptogenic liver tumours. They have been induced in the livers of rats (Stewart *et al.*, 1980; Williams, 1980), mice (Vesselinovitch *et al.*, 1985; Pitot, 1990), hamsters (Stenbäck *et al.*, 1986), fish (Hinton *et al.*, 1988) and monkeys (Ruebner *et al.*, 1976) and in turkey ova (Enzmann *et al.*, 1992).

HAF can be identified grossly but are usually diagnosed microscopically, either in conventional haematoxylin- and eosin-stained sections (Stewart *et al.*, 1980), or by histochemical techniques for visualization of the abnormalities described above. The development of HAF in rats can be monitored by measuring the activity of γ-glutamyltransferase in blood (Remandet *et al.*, 1984). HAF are generally quantified as number per square centimetre of the section area studied or as number per volume of liver.

Of the agents that have carcinogenic activity in liver, DNA-reactive carcinogens generally rapidly induce HAF, even within two to three weeks under some conditions (Williams, 1980, 1982; Dragan & Pitot, 1992). Epigenetic agents enhance the development of pre-existing spontaneous or chemically-induced HAF, but longer exposure is generally required to produce an increase (Kitigawa & Sugano, 1978; Pitot *et al.*, 1978; Watanabe & Williams, 1978; Williams, 1982; Dragan & Pitot, 1992). Thus, induction of HAF can be used to detect DNA-reactive hepatocarcinogens, while enhancement of HAF can be used to detect epigenetic agents with liver tumour-promoting activity (Williams, 1982; Tsuda *et al.*, 1984a; Bannasch, 1986a; Williams, 1989; Dragan *et al.*, 1991; Hirota & Williams, 1991). Inhibition of the induction of HAF by carcinogens is used to study anticancer agents (Maeura *et al.*, 1984; Tsuda *et al.*, 1984b; Mori *et al.*, 1986).

Preneoplastic lesions in the skin

Skin was the tissue used in the first experimental induction of cancer by a chemical, reported in 1915 by Yamagiwa and Ichikawa. The pioneering studies on skin carcinogenesis of Friedewald and Rous (1944) and Berenblum and Shubik (1947) introduced the concept of two stages of carcinogenesis — initiation and promotion — which has been the dominant paradigm since then (Boutwell, 1974; Slaga *et al.*, 1981), although some have questioned its validity in explaining the carcinogenic process (Iversen, 1995). Nevertheless, the pathogenesis of skin cancer involves multiple stages, including epidermal hyperplasia, epidermal dysplasia and benign papillomas, leading to malignant squamous-cell carcinomas (Berenblum, 1974; Stenbäck, 1978a; Kligman & Kligman, 1981; DiGiovanni, 1992; Yuspa, 1994).

The preneoplastic lesions are epidermal hyperplasia (Stenbäck, 1978a; Kligman & Kligman, 1981) and epidermal dysplasia (Stenbäck, 1978a). Epithelial papillomas, also called papillary hyperplasia or early papilloma, equally may be considered to be preneoplastic. Epidermal hyperplasia consists of increased layers of squamous epithelial cells with a normal appearance (Bogovski, 1979). Outgrowths may form above the surface. Epidermal hyperplasia can progress to epithelial papillomas, but it is induced by agents that are not carcinogenic and do not induce neoplasia; its preneoplastic nature may thus be questioned. Epidermal dysplasia is characterized by cellular atypia and a variable degree of hyperplasia. It is induced almost exclusively by carcinogenic agents, including solar radiation, but may regress upon cessation of exposure. Epithelial papillomas are grossly visible, protruding lesions composed predominantly of squamous epithelial cells. They may show atypia and dyskeratosis. They are characterized by a high rate of proliferation (Glick *et al.*, 1993) but can regress upon cessation of exposure (Shubik, 1950; Stenbäck, 1978b; Iversen, 1982), indicating that these morphologi-

cally tumour-like lesions are not fully neoplastic. Epithelial papillomas are diploid when they first emerge (Aldaz *et al.*, 1988), but they have mutations in the Ha c-*ras* gene (Balmain *et al.*, 1984). Nevertheless, they are precursors of squamous-cell carcinomas, which also may develop *ab initio* (Hennings *et al.*, 1983). The site of origin of epidermal hyperplasia and dysplasia and epithelial papillomas is the epidermis of the skin, specifically the squamous epithelium (Yuspa, 1994).

The superficial location of the skin allows visual detection of early lesions. Epidermal hyperplasia and dysplasia are identified microscopically and epithelial papillomas, grossly or microscopically. The latter are quantified by their incidence or sometimes number per animal.

In general, mice are more sensitive than rats or hamsters to skin carcinogenesis, and the induction of skin tumours in mice has been used as a bioassay for carcinogens (Weisburger & Williams, 1984); the SENCAR stock of mice is used predominantly, as it was bred for sensitivity to skin tumour induction (Slaga & Nesnow, 1985).

Preneoplastic lesions in the lung

Nodular lesions, commonly referred to as adenomas, were first induced in mouse lung by painting tar onto the skin (Murphy & Sturm, 1925). These lesions are considered to be bronchoalveolar neoplasms (Schüller, 1989). Tumours of the bronchial tree were produced by intratracheal instillation of polycyclic aromatic hydrocarbons into hamsters (Della Porta *et al.*, 1958), rats (Pylev, 1961; Shabad, 1962) and mice (Nettesheim & Hammons, 1971). This approach was refined by Saffiotti *et al.* (1968), who introduced the carcinogen attached to haematite. Bronchoalveolar adenomas were produced in hamsters by *N*-nitrosodiethylamine (Reznik-Schüller, 1976). Subsequent research with these models showed that the development of lung neoplasms proceeds through a phase of hyperplasia (Nettesheim, 1972; Shimkin & Stoner, 1975; Schüller, 1989).

In the mouse, general hyperplasia of alveolar cells precedes the development of focal hyperplasia and nodular lesions (Kimura, 1971; Kauffman, 1974). Some investigators have considered all such proliferative lesions to be neoplastic (Rehm & Ward, 1989; Foley *et al.*, 1991; Belinsky *et al.*, 1992). The alveolar hyperplasia progresses to benign-looking adenoma and thence to carcinoma (Kimura, 1971; Shimkin & Stoner, 1975). In the hamster, squamous metaplasia (Schreiber *et al.*, 1975; Stenbäck, 1977) and hyperplastic areas in the basal regions of the bronchial epithelium (Stenbäck, 1977; Schüller, 1989) precede the development of neoplasms.

The hyperplasia in mice consists of proliferation of type II epithelial cells along intact alveolar septae (Foley *et al.*, 1991). The adenomas are comprised of closely packed columns of cuboidal and columnar cells separated by a sparse stroma (Stewart *et al.*, 1979). The cells are arranged in acini, often with papillary formations. Hamster bronchial hyperplasia consists of increased numbers of basal cells, forming a multilayered epithelium (Mohr, 1982).

Adenomas, and presumably antecedent lesions, arise in mice either from type II cells of the alveolar epithelium (also referred to as granular pneumocytes: Grady & Stewart, 1940; Stewart *et al.*, 1979; Witschi & Haschek, 1983) or bronchiolar Clara cells (Kauffman, 1981). In the hamster, hyperplasia of the bronchial epithelium arises from basal cells (Mohr, 1982; Schüller, 1989). Adenomas occur spontaneously, especially in some strains of mice. They have been induced in both mice (Shimkin & Stoner, 1975) and rats (Schüller, 1987), and hyperplasia of the bronchial epithelium has been induced in hamsters (Mohr, 1982; Schüller, 1989). The induction of pulmonary adenomas in strain A mice has been used as a bioassay for carcinogens (Weisburger & Williams, 1984; Stoner & Shimkin, 1985).

In mice, 85% of hyperplastic lesions induced by a nitrosamine had K-*ras* mutations (Belinsky *et al.*, 1992). Small adenomas (1–2 mm) in strain A mice, 1–2 mm, were found to have a normal number of chromosomes, whereas larger lesions had abnormal numbers (Di Paolo, 1965).

Only 49% of mouse adenomas less than 1 mm in diameter were transplantable, whereas 100% of those greater than 3 mm could be transplanted (Klein, 1957). In another study, only 11% of adenomas were transplantable (DiPaolo, 1965).

Mouse lung nodules (presumably adenomas) can be quantified by gross examination or under a dissecting microscope, which permits identifica-

tion of lesions of about 1 mm in diameter. Otherwise, conventional microscopy is used to quantify adenomas. Nodules are usually quantified as number per animal.

Preneoplastic lesions in the mammary gland

Mammary tumours were reported in mice after application of 3-methylcholanthrene in 1936 by Maisin and Coolen and in 1941 by Engelbreth-Holm. The induction of mammary adenocarcinomas in female rats by administration of aromatic amines was reported in 1944 by Bielschowsky, and Shay and coworkers in 1949 induced these lesions with a polycyclic aromatic hydrocarbon. Study of the pathogenesis of experimental mammary gland cancer was greatly facilitated by the discovery of Huggins and coworkers (1959, 1961) of the rapid induction of mammary carcinoma in female Sprague-Dawley rats by 7,12-dimethylbenz[*a*]-anthracene administered at 50 days of age. Other useful rat models were introduced subsequently (Gullino *et al.*, 1975). Considerable research has since documented that chemically induced mammary tumours develop in mice and rats by a multistep process involving previous hyperplasia (Russo & Russo, 1987, 1996). The process is exquisitely dependent upon host factors (Welsch, 1985).

Preneoplastic lesions in mice include hyperplastic alveolar nodules (DeOme *et al.*, 1959; Faulkin, 1966) or alveolar bud hyperplasia (Russo & Russo, 1996), and intraductal proliferations (Russo & Russo, 1996); rats develop hyperplastic nodules (Beuving *et al.*, 1968) and ductal hyperplasia (Fisher *et al.*, 1975; Sinha & Dao, 1975) or intraductal proliferation (Russo & Russo, 1987).

Hyperplastic alveolar nodules are composed of up to hundreds of grouped, small, round alveoli, each surrounded by connective tissue and lined by small, dark cuboidal epithelium (Squartini, 1979). The sequence appears to be hyperplastic lesions, adenomas and adenocarcinomas. In ductal hyperplasia or intraductal proliferation in rats, ductal cells are enlarged, irregular in shape and increased in number, budding into the ductal lumen (Russo & Russo, 1987) or end buds (Haslam & Bern, 1977; Russo & Russo, 1996). The sequence appears to be ductal hyperplasia, carcinoma *in situ* and carcinoma. The lesions develop in mice in the terminal mammary ductules and in young rats in the terminal end buds (Beuving *et al.*, 1967; Russo & Russo, 1987) or the mammary ducts (Fisher *et al.*, 1975; Sinha & Dao, 1975), which are the main target in older animals (Russo & Russo, 1987).

The preneoplastic lesions in mouse mammary glands are mostly diploid but occasionally aneuploid (Banerjee & DeOme, 1963). Advanced hyperplasia induced in rats by 3-methylcholanthrene shows cytogenetic alterations. Ductal hyperplasia in rats is clearly a proliferative lesion, as mitoses are seen (Fisher *et al.*, 1975). In rats, ductal hyperplasia progresses to adenocarcinoma (Fisher *et al.*, 1975; Sinha & Dao, 1975).

Hyperplastic alveolar nodules frequently give rise to neoplasms after transplantation (DeOme *et al.*, 1959; Medina, 1976). Rat hyperplastic nodules and advanced hyperplasias are also transplantable (Beuving, 1968; Fisher *et al.*, 1975).

Hyperplastic alveolar nodules occur spontaneously in mice with a high background of mammary tumours, and this lesion, or alveolar bud hyperplasia, and intraductal proliferation have been induced in mice (DeOme *et al.*, 1959; Medina, 1976) and rats (Beuving, 1968).

Hyperplastic alveolar nodules are identified in whole-mount preparations stained with haematoxylin and examined under a dissecting microscope (Beuving *et al.*, 1967) or in conventional microscopic preparations. The superficial location of the mammary glands allows detection of large lesions by palpation. The production of hyperplastic alveolar lesions in mice was proposed by Faulkin (1966) as a measure of the effectiveness of a chemical as a carcinogenic agent. The rapid induction of mammary tumours in susceptible rat strains has been used as a bioassay for carcinogens (Weisburger & Williams, 1984; McCormick & Moon, 1985).

Preneoplastic lesions in the colon

Study of the pathogenesis of colon cancer became possible after the report in 1952 by Walpole and coworkers that 4-aminobiphenyl, and especially the 2′,3-dimethyl derivative, efficiently induced colon cancer in rats. Subsequently, other model carcinogens were identified (Pozharisski *et al.*, 1979; Weisburger & Fiala, 1983), notably 1,2-di-

methylhydrazine (Druckrey *et al.*, 1967), which induces colon cancer in rats, mice and hamsters. Work with such models indicated that experimental intestinal carcinoma develops *ab initio* and does not involve precursor adenomas (Pozharisski *et al.*, 1979). Later, foci of mucosal dysplasia (Kimura *et al.*, 1984) and aberrant crypt foci (ACF; Bird, 1987; McLellan & Bird, 1988) were identified as putative precursors of colon cancer which are specifically induced by colon carcinogens (McLellan *et al.*, 1991a,b).

ACF are focal lesions composed of one to several enlarged crypts lined with thicker, more intensely staining epithelial cells (McLellan & Bird, 1988). They can be visualized macroscopically but do not distort the architecture of the mucosa. The site of origin of ACF is the colonic mucosa, specifically mucosal epithelial cells.

Phenotypic alterations in ACF include enzymatic changes such as decreased hexosamidase activity (Pretlow *et al.*, 1990). ACF also have alterations in nuclear DNA content (Kimura *et al.*, 1984) and mutations in the K-*ras* oncogene (Stopera *et al.*, 1992; Vivona *et al.*, 1993).

The growth of ACF is reported to correlate with the yield of adenocarcinomas (Pretlow *et al.*, 1992; Zhang *et al.*, 1992; Magnuson *et al.*, 1993), although no correlation between the number of ACF per rat and the incidence of adenocarcinomas was found in some studies (Hardman *et al.*, 1991). ACF have been induced in mice (McLellan & Bird, 1988) and rats (Barrow *et al.*, 1990).

ACF are identified in methylene blue-stained whole-mount preparations under a dissecting microscope. They are usually quantified as number per animal or per colon. The number of ACF per animal is used as an assay for initiators of colon cancer (Bird *et al.*, 1989) and the number of crypts per focus (multiplicity) as a measure of promotion (Corpet *et al.*, 1990; Zhang *et al.*, 1992; Magnuson *et al.*, 1993). Inhibition of the development of ACF can be used to assess anticancer activity (McLellan & Bird, 1991; Weisburger *et al.*, 1994; Wargovich *et al.*, 1996).

Preneoplastic lesions in the kidney

The study of experimental renal carcinogenesis started with the work of Magee and Barnes (1962). Most of the studies were conducted in rats given nitrosamines (Hard, 1990), although other models have been developed (Dees *et al.*, 1976). The pathogenesis of rat cortical epithelial tumours involves a proliferative sequence (Hard & Butler, 1989; Nogueira *et al.*, 1993). Proliferative tubules lined with an irregular epithelium (Magee & Barnes, 1962), foci of atypical tubular hyperplasia (Reuber, 1974) and phenotypically altered tubules (Hard *et al.*, 1995) have been described as preneoplastic lesions in rats (Lipsky & Trump, 1988) and mice (Lombard *et al.*, 1974). In hamsters, areas of dysplasia have been described (Horning & Whittick, 1954).

Atypical tubular hyperplasia is characterized by cells with cytoplasm that stains as clear, basophilic, acidophilic, oncolytic or chromophobic (Reuber, 1974; Bannasch *et al.*, 1986; Dietrich & Swenberg, 1991). It consists of one or several single or multilayered tubules of altered cells. The lesion does not exceed 10 times the size of surrounding normal tubules and does not compress the surrounding parenchyma.

The origin of atypical tubular hyperplasia in rats has been identified as the distal (Reuber, 1974) or proximal convoluted tubules (Ohmori *et al.*, 1982). In hamsters, areas of dysplasia were observed in proximal tubule segments (Horning & Whittick, 1954).

Clear-cell lesions store glycogen (Dietrich & Swenberg, 1991) and show various histochemically detectable alterations in carbohydrate metabolism (Bannasch *et al.*, 1986). They also have reduced γ-glutamyltransferase activity (Ohmori *et al.*, 1982). In hamsters, the lesion is proliferative (Goldfarb & Pugh, 1990).

Atypical tubular hyperplasia in rats, consisting of basophilic-cell tubules, is regarded as a precursor of renal-cell carcinoma developing from the epithelia of the proximal tubules, whereas clear-cell tubules have been considered to be precursors of renal-cell carcinoma developing from the epithelium of the collecting duct (Bannasch *et al.*, 1986). In the hamster, the lesion progresses to adenocarcinomas (Horning & Whittick, 1954; Goldfarb & Pugh, 1990).

Atypical tubular hyperplasia occurs spontaneously in rats but has also been induced in rats (Hard *et al.*, 1995) and mice (Lombard *et al.*,

1974). The lesion is detected microscopically and is usually quantified as incidence.

Preneoplastic lesions in the pancreas

Neoplasms were induced experimentally in the pancreas of rats with 4-hydroxyaminoquinoline 1-oxide by Hayashi and Hasegawa in 1971. Pour *et al.* (1974) induced pancreatic cancer in hamsters with 2,2'-dihydroxy-di-*n*-propylnitrosamine, Reddy *et al.* (1974) in guinea-pigs with *N*-methyl-*N*-nitrosourea, Dissin *et al.* (1975) in rats with implantation of 7,12 dimethylbenz[*a*]anthracene and Longnecker and Curphey (1975) in rats with azaserine. Studies with these models established that carcinogenesis in the pancreas is a multistep process (Longnecker *et al.*, 1984).

In the rat model, atypical acinar-cell foci or atypical acinar-cell nodules have been described as preneoplastic lesions (Hayashi *et al.*, 1972; Shinozuka *et al.*, 1976; Rao *et al.*, 1982; Mori *et al.*, 1983b; Roebuck *et al.*, 1984). In the hamster model, pseudoductular or tubular structures (Pour & Hauser, 1989) and epithelial atypia (Moore *et al.*, 1983) are considered to be preneoplastic.

Atypical acinar-cell foci in rats are microscopic lesions composed of cells that stain acidophilically or basophilically. The basophilic foci are composed of cells with a basophilic cytoplasm and large, irregular nuclei located parabasally. The cells are arranged in acini of normal size. The acidophilic foci are composed of cells with an acidophilic cytoplasm and round or oval nuclei with prominent nucleoli. The cells are arranged in acini which are larger than normal. Acinar-cell nodules are larger lesions, and lesions greater than 3 mm in diameter are diagnosed as adenomas (Longnecker & Millar, 1990). Microscopically, they are composed of cells similar to those in atypical acinar-cell foci and compress the adjacent parenchyma.

The foci in rats are derived from acinar cells, whereas the lesions in hamsters have been identified as originating from ducts (Takahashi *et al.*, 1980) or centroacinar cells (Flaks, 1984). The cells of basophilic atypical acinar-cell foci in rats have few zymogen granules, whereas the cytoplasm of acidophilic cells is filled with zymogen granules (Rao *et al.*, 1982). Acinar-cell foci and nodules in rats also have histochemical abnormalities, such as reduced iron storage (Mori *et al.*, 1983b) and reduced γ-glutamyltransferase activity (Mori *et al.*, 1983b; Faribault *et al.*, 1987). Rat acinar-cell lesions also have an abnormal DNA content (Tanaka *et al.*, 1984). Acidophilic foci in rats have a high rate of cell proliferation (Rao *et al.*, 1982), as do the pseudoductular structures in hamsters (Moore *et al.*, 1983).

Acidophilic foci in rats are considered to progress to acinar-cell tumours (Rao *et al.*, 1982; Boorman & Eustis, 1984). The pseudoductular proliferations in hamsters have been suggested to progress to regions of epithelial atypia (dysplastic lesions) and to carcinomas (Moore *et al.*, 1983). The hamster acinar-cell lesions progressively lose differentiation and, with proliferation, evolve into neoplasms (Flaks, 1984; Pour & Hauser, 1989).

Atypical acinar-cell foci both occur spontaneously in rats and have been induced (Longnecker *et al.*, 1984). Pseudoductular structures have been induced in hamsters (Pour & Hauser, 1989).

Pancreatic lesions are identified microscopically. They can be quantified as number per square centimetre.

Preneoplastic lesions in other organs

Putative preneoplastic lesions have been described in other tissues, including the urinary bladder (Cohen *et al.*, 1976; Hicks & St John Wakefield, 1976; Ito, 1976) and stomach, but either studies have not been reported in the detail available for the tissues reviewed above, or the models have not been extensively applied to assessment of the carcinogenic activity of chemicals. In a number of other tissues, however, no putative preneoplastic lesions have been identified.

Conclusion

In all well-studied experimental models of chemical carcinogenesis, focal proliferative lesions composed of abnormal cells appear before the occurrence of neoplasms, although precursors have not been documented for some tumour types. The recognized precursor lesions occur spontaneously in older animals and in response to carcinogens in younger animals, usually before the development of significant numbers of spontaneous lesions. The preneoplastic nature of these lesions is reflected by

phenotypic abnormalities similar to those in neoplasms, genotypic abnormalities and the capacity of some to give rise to neoplasms after transplantation (Table 1). The latter phenomenon may indicate, however, that some of these lesions harbour neoplastic cells.

The number of preneoplastic lesions generally exceeds that of neoplasms, indicating that not all of the lesions are committed to progression. In several tissues, such as liver and colon, preneoplastic lesions can be identified easily by special staining techniques, and they have been used extensively as surrogates for neoplasms to assess the carcinogenic activity of chemicals. In other tissues, such as mouse skin, mouse lung and rat mammary gland, the progression of precursor lesions to tumour formation is so rapid that tumours are used as the end-point, although their neoplastic nature is not always unequivocal and some of the lesions may in fact be preneoplastic. Nevertheless, the detection of preneoplastic or incipient neoplastic lesions of the type discussed above serves as a facile indicator of the carcinogenic activity of the agent that induced them.

The advantages of monitoring preneoplastic lesions as an index of carcinogenic activity are the short time required, the sensitivity, due the multiplicity of such lesions, and the absence of age-related pathological lesions. The disadvantages are that recognized preneoplastic lesions have not been established for every target organ of the carcinogenicity of chemicals, and some models have not been studied extensively.

So far, preneoplastic lesions have been studied only in conventional strains of rodents. In principle, they also can be identified in transgenic animals, thereby accelerating even further the detection of carcinogenic effects.

References

Aldaz, C.M., Conti, C.J., Larcher, F., Trono, D., Roop, D.R., Chesner, J., Whitehead, T. & Slaga, T.J. (1988) Sequential development of aneuploidy, keratin modification and γ-glutamyltransferase expression in mouse skin papillomas. *Cancer Res.*, **48**, 3253–3257

Balmain, A., Ramsden, M., Bowden, G.T. & Smith, J. (1984) Activation of the mouse cellular Harvey-*ras* gene in chemically induced benign skin papillomas. *Nature*, **307**, 658–660

Banerjee, M.R. & DeOme, K.B. (1963) Chromosomes in normal, preneoplastic, and neoplastic tissues of the mammary glands of C3H/Crgl female mice. *Cancer Res.*, **23**, 546–550

Bannasch, P. (1968) The cytoplasm of hepatocytes during carcinogenesis. Electron and light microscopical investigations of the nitrosomorpholine-intoxicated rat liver. *Recent Results Cancer Res.*, **19**, 1–100

Bannasch, P. (1986a) Preneoplastic lesions as endpoints in carcinogenicity testing. I. Hepatic preneoplasia. *Carcinogenesis*, **7**, 689–695

Bannasch, P. (1986b) Preneoplastic lesions as end points in carcinogenicity testing. II. Preneoplasia in various non-hepatic tissues. *Carcinogenesis*, 7, 849–852

Bannasch, P. & Zerban, H. (1990) Tumours of the liver. In: Turusov, V. & Mohr, U., eds, *Pathology of Tumours in Laboratory Animals*, Volume 1, *Tumours of the Rat* (IARC Scientific Publications No. 99), Lyon, IARC, pp. 208–212

Bannasch, P., Hacker, H.J., Tsuda, H. & Zerban, H. (1986) Aberrant carbohydrate metabolism and metamorphosis during renal carcinogenesis. *Adv. Enzyme Regul.*, **25**, 279–296

Barrow, B.J., O'Riordan, M.A., Stellato, T.A., Calkins, B.M. & Pretlow, T.P. (1990) Enzyme-altered foci in colons of carcinogen-treated rats. *Cancer Res.*, **50**, 1911–1916

Bauer-Hofmann, R., Buchmann, A., Mahr, J., Kress, S. & Schwarz, M. (1992) The tumour promoters dieldrin and phenobarbital increase the frequency of c-Ha-*ras* wild-type, but not c-Ha-*ras* mutated focal liver lesions in male C3H/He mice. *Carcinogenesis*, **13**, 477–481

Becker, F.F. (1976) Sequential phenotypic and biochemical alterations during chemical hepatocarcinogenesis. *Cancer Res.*, **36**, 2563–2566

Belinsky, S.A., Devereux, T.R., Foley, J.F., Maronpot, R.R. & Anderson, M.W. (1992) Role of the alveolar type II cell in the development and progression of pulmonary tumours induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in the A/J mouse. *Cancer Res.*, **52**, 3164–3173

Berenblum, I. (1974) *Carcinogenesis as a Biological Problem* (*Frontiers of Biology*, Vol. 34), Amsterdam, North-Holland Publishing Company

Berenblum, I. & Shubik, P. (1947) A new, quantitative approach to the study of the stages of chemical car-

cinogenesis in the mouse's skin. *Br. J. Cancer*, **1**, 383–391

Beuving, L.J. (1968) Mammary tumour formation within out-growths of transplanted hyperplastic nodules from carcinogen-treated rats. *J. natl Cancer Inst.*, **40**, 1287–1291

Beuving, L.J., Faulkin, L.J., Jr, De Ome, K.B. & Bergs, V.V. (1967) Hyperplastic lesions in the mammary glands of Sprague-Dawley rats after 7,12-dimethylbenz[*a*]anthracene treatment. *J. natl Cancer Inst.*, **39**, 423–430

Bielschowsky, F. (1944) Distant tumours produced by 2-amino- and 2-acetylaminofluorene. *Br. J. exp. Pathol.*, **25**, 1–4

Bird, R.P. (1987) Observation and quantification of aberrant crypts in murine colon treated with a colon carcinogen: Preliminary findings. *Cancer Lett.*, **37**, 147–151

Bird, R.P., McLellan, E. & Bruce, W.R. (1989) Aberrant crypts, putative precancerous lesions, in the study of the role of diet in the aetiology of colon cancer. *Cancer Surv.*, **8**, 189–200

Bogovski, P. (1979) Tumours of the skin. In Turusov, V.S., ed., *Pathology of Tumours in Laboratory Animals*, Volume II, *Tumours of the Mouse* (IARC Scientific Publications No. 23), Lyon, IARC, p. 16

Boorman, G.A. & Eustis, S.L. (1984) Proliferative lesions of the exocrine pancreas in male F344/N rats. *Environ. Health Perspectives*, **56**, 213–217

Boutwell, R.K. (1974) The function and mechanism of promoters of carcinogenesis. *CRC crit. Rev. Toxicol.*, **2**, 419–443

Cameron, R., Sweeney, G.D., Jones, K., Lee, G. & Farber, E. (1976) A relative deficiency of cytochrome P-450 and aryl hydrocarbon (benzo[a]pyrene) hydroxylase in hyperplastic nodules induced by 2-aminoacetylfluorene in rat liver. *Cancer Res.*, **36**, 3888–3893

Cohen, S.M., Jacobs, J.B., Arai, M., Johansson, S. & Friedell, G.H. (1976) Early lesions in experimental bladder cancer: Experimental design and light microscopic findings. *Cancer Res.*, **36**, 2508–2511

Corpet, D.E., Stamp, D., Medline, A., Minkin, S., Archer, M.C. & Bruce, W.R. (1990) Promotion of colonic microadenoma growth in mice and rats fed cooked sugar or cooked casein and fat. *Cancer Res.*, **50**, 6955–6958

Daoust, R. & Calamai, R. (1971) Hyperbasophilic foci as sites of neoplastic transformation in hepatic parenchyma. *Cancer Res.*, **31**, 1290–1296

Daoust, R. & Molnar, F. (1964) Cellular populations and mitotic activity in rat liver parenchyma during azo dye carcinogenesis. *Cancer Res.*, **24**, 1898–1090

Dees, J.H., Reuber, M.D. & Trump, B.F. (1976) Adenocarcinoma of the kidney. I. Ultrastructure of renal adenocarcinomas induced in rats by N-(4'-fluoro-4-biphenylyl)acetamide. *J. natl Cancer Inst.*, **57**, 779–794

Della Porta, G., Kolb, L. & Shubik, P. (1958) Induction of tracheobronchial carcinomas in the Syrian golden hamster. *Cancer Res.*, **18**, 592–597

DeOme, K.B., Faulkin, L.J., Jr, Bern, H.A. & Blair, P.B. (1959) Development of mammary tumours from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. *Cancer Res.*, **19**, 515–520

Dietrich, D.R. & Swenberg, J.A. (1991) Preneoplastic lesions in rodent kidney induced spontaneously or by non-genotoxic agents: Predictive nature and comparison to lesions induced by genotoxic carcinogens. *Mutat. Res.*, **248**, 239–260

DiGiovanni, J. (1992) Multistage carcinogenesis in mouse skin. *Pharmacol. Ther.*, **54**, 63–128

Di Paolo, J.A. (1965) Chromosome patterns in mouse lung tumours. *J. natl Cancer Inst.*, **34**, 337–343

Dissin, J., Mills, L.R., Mainz, D.L., Black, O., Jr & Webster, P.D., III (1975) Experimental induction of pancreatic adenocarcinoma in rats. *J. natl Cancer Inst.*, **55**, 857–864

Dragan, Y.P. & Pitot, H.C. (1992) The role of the stages of initiation and promotion in phenotypic diversity during hepatocarcinogenesis in the rat. *Carcinogenesis*, **13**, 477–481

Dragan, Y.P., Rizvi, T., Xu, Y.-H., Hully, J.R., Bawa, N., Campbell, H.A., Maronpot, R.R. & Pitot, H.C. (1991) An initiation-promotion assay in rat liver as a potential complement to the two year carcinogenesis bioassay. *Fundam. appl. Toxicol.*, **16**, 525–547

Drinkwater, N.R., Hanigan, M.H. & Kemp, C.J. (1990) Genetic and epigenetic promotion of murine hepatocarcinogenesis. In: Stevenson, D.E., McClain, J.A., Popp, R.M., Slaga, T.J. Ward, J.M. & Pitot, H., eds, *Mouse Liver Carcinogenesis: Mechanisms and Species Comparisons*, New York, Wiley-Liss, pp. 163–176

Druckrey, H., Preussman, R., Matzkies, F. & Ivankovic, S. (1967) Selective induction of intestinal cancer in

rats by 1,2-dimethylhydrazine. *Naturwissenschaften*, **54**, 285–286 (in German)

Engelbreth-Holm, J. (1941) Acceleration of development of mammary carcinomas in mice by methylcholanthrene. *Cancer Res.*, **1**, 109–112

Enzmann H., Kaliner, G., Watta-Gebert, B. & Löser, E. (1992) Foci of altered hepatocytes induced in embryonal turkey liver. *Carcinogenesis*, **13**, 943–946

Farber, E. (1973) Hyperplastic liver nodules. *Meth. Cancer Res.*, **7**, 345–375

Farber, E. & Cameron, R. (1980) The sequential analysis of cancer development. *Adv. Cancer Res.*, **31**, 125–226

Farber, E., Parker, S. & Gruenstein, M. (1976) The resistance of putative premalignant liver cell populations, hyperplastic nodules, to the acute cytotoxic effects of some carcinogens. *Cancer Res.*, **36**, 3879–3887

Faribault, G., Wiebkin, P., Hamilton, J.W., Longnecker, D.S. & Curphey, T.J. (1987) γ-Glutamyltransferase activity in atypical acinar cell nodules of rat pancreas. *Toxicol. appl. Pharmacol.*, **88**, 338–345

Faulkin, L.J., Jr (1966) Hyperplastic lesions of mouse mammary glands after treatment with 3-methylcholanthrene. *J. natl Cancer Inst.*, **36**, 289–298

Fausto, N., Lemire, J.M. & Shiojiri, N. (1992) Oval cells in liver carcinogenesis: Cell lineages in hepatic development and the identification of facultative stem cells in normal liver. In: Sirica, A.E., ed., *The Role of Cell Types in Hepatocarcinogenesis*, Boca Raton, FL, CRC Press, pp. 89–108

Firminger, H.I. (1955) Histopathology of carcinogenesis and tumours of the liver in rats. *J. natl Cancer Inst.*, **15**, 1427–1442

Fisher, E.R., Shoemaker, R.H. & Sabnis, A. (1975) Relationship of hyperplasia to cancer in 3-methylcholanthrene-induced mammary tumorigenesis. *Lab. Invest.*, **33**, 33–42

Flaks, B. (1984) Histogenesis of pancreatic carcinogenesis in the hamster: Ultrastructural evidence. *Environ. Health Perspectives*, **56**, 187–203

Foley, J.F., Anderson, M.W., Stoner, G.D., Gaul, B.W., Hardisty, J.F. & Maronpot, R.R. (1991) Proliferative lesions of the mouse lung: Progression studies in strain A mice. *Exp. Lung Res.*, **17**, 157–168

Foulds, L. (1969) *Neoplastic Development*, Vol. 1, London, Academic Press

Friedewald, W.F. & Rous, P. (1944) Initiating and promoting elements in tumour production; analysis of effects of tar, benzpyrene, and methylcholanthrene on rabbit skin. *J. exp. Med.*, **80**, 101–126

Galand, P., Jacobovitz, D. & Alexandre, K. (1988) Immunohistochemical detection of c-Ha-*ras* oncogene p21 product in pre-neoplastic and neoplastic lesions during hepatocarcinogenesis in rats. *Int. J. Cancer*, **41**, 155–161

Gebhardt, R. & Williams, G.M. (1995) Glutamine synthetase and hepatocarcinogenesis. *Carcinogenesis*, **16**, 2673–1681

Gebhardt, R., Tanaka, T. & Williams, G.M. (1989) Glutamine synthetase heterogenous expression as a marker for the cellular lineage of preneoplastic and neoplastic liver populations. *Carcinogenesis*, **10**, 1917–1923

Glick, A.B., Kulkarni, A.B., Tennenbaum, T., Hennings, H., Flanders, K.C., O'Reilly, M., Sporn, M.B., Karlsson, S. & Yuspa, S.H. (1993) Loss of TGF-β expression in mouse epidermis and epidermal tumors is associated with hyperproliferation and a high risk for malignant conversion. *Proc. natl Acad. Sci. USA*, **90**, 6076–6080

Goldfarb, S. & Pugh, T.D. (1990) Morphology and anatomic localization of renal microneoplasms and proximal tubule dysplasias induced by four different estrogens in the hamster. *Cancer Res.*, **50**, 113–119

Goldfarb, S. & Pugh, T.D. (1992) Histogenesis and pathobiology of mouse hepatocellular neoplasms. In: Sirica A.E., ed., *The Role of Cell Types in Hepatocarcinogenesis*, Boca Raton, FL, CRC Press, pp. 55–70

Gössner, W. & Friedrich-Freska, H. (1964) Histochemical investigation of glucose-6-phosphatase in the rat liver during nitrosamine carcinogenesis. *Z. Naturforsch*, **19**, 862–863 (in German)

Grady H.G. & Stewart, H.L. (1940) Histogenesis of induced pulmonary tumours in strain A mice. *Am. J. Pathol.*, **16**, 417–432

Gullino, P.M., Pettigrew, H.M. & Grantham, F.H. (1975) *N*-Nitrosomethylurea as mammary gland carcinogen in rats. *J. natl Cancer Inst.*, **54**, 401–414

Hacker, H.J., Moore, M.A., Mayer, D. & Bannasch, P. (1982) Correlative histochemistry of some enzymes of carbohydrate metabolism in preneoplastic and neoplastic lesions in the rat liver. *Carcinogenesis*, **3**, 1265–1272

Hard, G.C. (1990) Tumours of the kidney, renal pelvis and ureter. In: Turusov, V. & Mohr, U, eds., *Pathology*

of Tumours in Laboratory Animals, Volume 1, *Tumours of the Rat*, 2nd Ed., (IARC Scientific Publications No. 99), Lyon, IARC, pp. 301–344

Hard, G.C. & Butler, W.H. (1989) The comparative pathology of dimethylnitrosamine-induced renal tumours in the rat. In: Schuller, H.M., ed., *Comparative Ultrastructural Pathology of Selected Tumours in Man and Animals*, Boca Raton, FL, CRC Press, pp. 97–132

Hard, G.C., Alden, C.L., Stula, E.F. & Trump, B.F (1995) Proliferative lesions of the kidney in rats. In: *Guides for Toxicologic Pathology*, Washington DC, Society of Toxicologic Pathologists/American Registry of Pathology/Armed Forces Institute of Pathology, pp. 1–19

Hardman, W.E., Cameron, I.L., Heitman, D.W. & Contreras, E. (1991) Demonstration of the need for end point validation of putative biomarkers: Failure of aberrant crypt foci to predict colon cancer incidence. *Cancer Res.*, **51**, 6388–6392

Haslam, S.Z. & Bern, H.A. (1977) Histopathogenesis of 7,12-dimethylbenz[a]anthracene-induced rat mammary tumours. *Proc. natl Acad. Sci. USA*, **74**, 4020–4024

Hayashi, Y & Hasegawa, T. (1971) Experimental pancreatic tumour in rats after intravenous injection of 4-hydroxyaminoquinoline 1-oxide. *Gann*, **62**, 329–330

Hayashi, Y., Furukawa, H., & Hasegawa, T. (1972) Pancreatic tumours in rats induced by 4-nitroquinoline-1-oxide derivatives. In: Nakahara, W., Takayama, S., Sugimura, T. & Odashima, S., eds, *Topics in Chemical Carcinogenesis*, Baltimore, University Park Press, pp. 53–65

Hennings, H., Shores, R., Wenk, M.L., Spangler, E.F., Tarone, R. & Yuspa, S.H. (1983) Malignant conversion of mouse skin tumors is increased by tumor initiators and unaffected by tumor promoters. *Nature*, **304**, 67–69

Hicks, R.M. & St John Wakefield, J. (1976) Membrane changes during urothelial hyperplasia and neoplasia. *Cancer Res.*, **36**, 2502–2507

Hinton, D.E., Couch, J.A., The, S.J. & Courtney, L.A. (1988) Cytological changes during progression of neoplasia in selected fish species. *Aquat. Toxicol.*, **11**, 77–112

Hirota, N. & Williams, G.M. (1979) The sensitivity and heterogeneity of histochemical markers for altered foci involved in liver carcinogenesis. *Am. J. Pathol.*, **95**, 317–328

Hirota, N. & Williams, G.M. (1982) Ultrastructural abnormalities in carcinogen-induced hepatocellular altered foci identified by resistance to iron accumulation. *Cancer Res.*, **42**, 2298–2309

Hirota, N. & Williams, G.M. (1991) Assessment of hepatocarcinogenesis by early indicators. In: Meeks, R.G., Harrison, S.D., Jr & Bull, R.J., eds, *Hepatotoxicology*, Boca Raton, FL, CRC Press, pp. 593–620

Horning, E.S. & Whittick, J.W. (1954) The histogenesis of stilbestrol-induced renal tumours in the male golden hamster. *Br. J. Cancer*, **8**, 451–457

Huggins, C., Briziarelli, G. & Sutton, H. (1959) Rapid induction of mammary carcinoma in the rat and the influence of hormones on the tumours. *J. exp. Med.*, **109**, 25–42

Huggins, C., Grand, L.C. & Brillantes, F.P. (1961) Mammary cancer induced by a single feeding of polynuclear hydrocarbons and its suppression. *Nature*, **189**, 205–207

Ito, N. (1976) Early changes caused by N-butyl-n-(4-hydroxybutyl)nitrosamine in the bladder epithelium of different animal species. *Cancer Res.*, **36**, 2528–2531

Iversen, O.H. (1982) Hairless mouse skin in two-stage chemical carcinogenesis. *Virchows Arch. (Cell Pathol.)*, **38**, 263–272

Iversen, O.H. (1995) Of mice and men: A critical reappraisal of the two-stage theory of carcinogenesis. *Crit. Rev. Oncogenesis*, **6**, 357–405

Kalengayi, M.M.R., Rochi, G. & Desmet, V.J. (1975) Histochemistry of gamma-glutamyl transpeptidase in rat liver during aflatoxin B1-induced carcinogenesis. *J. natl Cancer Inst.*, **55**, 579–588

Karasaki, S. (1969) The fine structure of proliferating cells in preneoplastic rat livers during azo-dye carcinogenesis. *J. Cell Biol.*, **40**, 322–335

Kauffman, S.L. (1974) Kinetics of alveolar epithelial hyperplasia in lungs of mice exposed to urethane. I. Quantitative analysis of cell populations. *Lab. Invest.*, **30**, 170–175

Kauffman, S.L. (1981) Histogenesis of the papillary Clara cell adenoma. *Am. J. Pathol.*, **103**, 174–180

Kaufman, W.K., Zhang, Y. & Kaufman, D.G. (1992) Association between expression of transforming growth factor-alpha and progression of hepatocellular foci to neoplasms. *Carcinogenesis*, **13**, 1481–1483

Kimura, K. (1971) Progression of pulmonary tumour in

mice. I. Histological studies of primary and transplanted pulmonary tumours. *Acta pathol. Jpn.*, **21**, 13–56

Kimura, O., Kaibara, N., Miyano, Y., Okamoto, T., Tamura, H., Yurugi, E. & Koga, S. (1984) Nuclear DNA content in dimethylhydrazine-induced colonic carcinoma and mucosal dysplasia in rats. *Cancer*, **53**, 1918–1922

Kitigawa, T. (1971) Histochemical analysis of hyperplastic lesions and hepatomas of the liver of rats fed 2-fluorenylacetamide. *Gann*, **62**, 207–216

Kitigawa, T. & Sugano, H. (1978) Enhancing effect of phenobarbital on the development of enzyme-altered islands and hepatocellular carcinomas initiated by 3'-methyl-4-(dimethylamino)azobenzene or diethylnitrosamine. *Gann*, **69**, 679–687

Klein, M. (1957) The transplantability and growth of urethan-induced pulmonary adenomas of the mouse. *Cancer Res.*, **17**, 655–658

Kligman, L.H. & Kligman, A.M. (1981) Histogenesis and progression of ultraviolet light-induced tumours in hairless mice. *J. natl Cancer Inst.*, **67**, 1289–1297

Krutovskikh, V.A., Oyamada, M. & Yamasaki, H. (1991) Sequential changes of gap-junctional intercellular communications during multistage rat liver carcinogenesis: Direct measurement of communication *in vivo*. *Carcinogenesis*, **12**, 1701–1706

Lipsky, M.M. & Trump, B.F. (1988) Chemically induced renal epithelial neoplasia in experimental animals. *Int. Rev. exp. Pathol.*, **30**, 357–383

Lombard, L.S., Rice, J.M. & Vesselinovitch, S.D. (1974) Renal tumours in mice: light microscopic observations of epithelial tumours induced by ethylnitrosourea. *J. natl Cancer Inst.*, **53**, 1677–1686

Longnecker, D.S. & Curphey, T.J. (1975) Adenocarcinoma of the pancreas in azaserine-treated rats. *Cancer Res.*, **35**, 2249–2257

Longnecker, D.S. & Millar, P.M. (1990) Tumours of the pancreas. In: Turusov, V. & Mohr, U., eds, *Pathology of Tumours in Laboratory Animals*, Volume 1, *Tumours of the Rat* (IARC Scientific Publications No. 99), Lyon, IARC, pp. 241–258

Longnecker, D.S., Wiebkin, P., Schaeffer, B.K. & Roebuck, B.D. (1984) Experimental carcinogenesis in the pancreas. *Int. Rev. exp. Pathol.*, **26**, 177–229

Maeura, Y., Weisburger, J.H. & Williams, G.M. (1984) Dose-dependent reduction of *N*-2-fluorenylacetamide-induced liver cancer and enhancement of bladder cancer in rats by butylated hydroxytoluene. *Cancer Res.*, **44**, 1604–1610

Magee, P.N. & Barnes, J.M. (1962) Induction of kidney tumours in the rat with dimethylnitrosamine (*N*-nitrosodimethylamine). *J. Pathol. Bacteriol.*, **84**, 19–31

Magnuson, B.A., Carr., I. & Bird, R.P. (1993) Ability of aberrant crypt foci characteristics to predict colonic tumour incidence in rats fed cholic acid. *Cancer Res.*, **53**, 4499–4504

Maisin, J. & Coolen, M.L. (1936) Carcinogenic potency of methylcholanthrene. *C.R. Soc. Biol. (Paris)*, **123**, 159–160 (in French)

Marceau, N., Blouin, M.-J., Noël, M., Török, N. & Loranger, A. (1992) The role of bipotential progenitor cells in liver ontogenesis and neoplasia. In: Sirica, A.E., ed., *The Role of Cell Types in Hepatocarcinogenesis*, Boca Raton, FL, CRC Press, pp. 121–150

Maruyama, H., Tanaka, T., Gebhardt, R., Berghem, L. & Williams, G.M. (1988) Decreased fibronectin in rat liver altered foci and adenomas induced by *N*-2-fluorenylacetamide. *Lab. Invest.*, **58**, 630–635

Matsuura, B., Taniguchi, Y. & Ohta, Y. (1994) Effect of antiandrogen treatment on chemical hepatocarcinogenesis in rats. *J. Hepatol.*, **21**, 187–193

McCormick, D.L. & Moon, R.C. (1985) Tumorigenesis of the rat mammary gland. In: Milman, H.A. & Weisburger, E.K., eds, *Handbook of Carcinogen Testing*, Park Ridge, NJ, Noyes Publications, pp. 215–229

McLellan, E.A. & Bird, R.P. (1988) Aberrant crypts: Potential preneoplastic lesions in the murine colon. *Cancer Res.*, **48**, 6187–6192

McLellan, E. & Bird, R.P. (1991) Effect of disulfiram on 1,2-dimethylhydrazine- and azoxymethane-induced aberrant crypt foci. *Carcinogenesis*, **12**, 969–972

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

McLellan, E.A., Medline, A. & Bird, R.P. (1991b) Dose response and proliferative characteristics of aberrant crypt foci — putative preneoplastic lesions in rat colon. *Carcinogenesis*, **12**, 2093–2098

Medina, D. (1976) Mammary tumorigenesis in chemical carcinogen-treated mice. VI. Tumour-producing capability of mammary dysplasias in BALB/cCrgl mice. *J. natl Cancer Inst.*, **57**, 1185–1189

Mohr, U. (1982) Tumours of the respiratory tract. In: Turusov, V.S. ed., *Pathology of Tumours in Laboratory Animals*, Volume III, *Tumours of the Hamster* (IARC Scientific Publications No. 34), Lyon, IARC, pp. 115–146

Montesano, R., Bartsch, H., Vainio, H., Wilbourn, J. & Yamasaki, H., eds (1986) *Long-term and Short-term Assays for Carcinogens: A Critical Appraisal* (IARC Scientific Publications No. 83), Lyon, IARC, pp. 85–102

Moore, M.A., Takahashi, M., Ito, N. & Bannasch, P. (1983) Early lesions during pancreatic carcinogenesis induced in Syrian hamster by DHPN or DOPN. I. Histologic, histochemical and radioautographic findings. *Carcinogenesis*, **4**, 431–437

Mori, H., Tanaka, T., Sugie, S., Takashashi, M. & Williams, G.M. (1982) DNA content of liver cell nuclei of N-2-fluorenylacetamide-induced altered foci and neoplasms in rats and human hyperplastic foci. *J. natl Cancer Inst.*, **69**, 1277–1282

Mori, H., Furuya, K. & Williams, G.M. (1983a) Enhanced survival and absence of progressive growth of transplanted rat liver altered eosinophilic foci and neoplastic nodules in phenobarbital-treated rats. *J. natl Cancer Inst.*, **71**, 849–854

Mori, , H., Tanaka, T., Takahashi, M. & Williams, G.M. (1983b) Exclusion of cellular iron and reduced gamma-glutamyl transpeptidase activity in rat pancreas acinar cell hyperplastic nodules and adenomas induced by azaserine. *Gann*, **74**, 497–501

Mori, H., Tanaka, T., Shima, H., Kuniyasu, T. & Takahashi, M. (1986) Inhibitory effect of chlorogenic acid on methylazoxymethanol acetate-induced carcinogenesis in large intestine and liver of hamsters. *Cancer Lett.*, **30**, 49–54

Mori, H., Ichida, T., Tanaka, T. & Williams, G.M. (1989) Pathological features of preneoplastic and neoplastic liver lesions in rodents and humans. In: Schuller, H.M., ed., *Comparative Ultrastructural Pathology of Selected Tumours in Man and Animals*, Boca Raton, FL, CRC Press, pp. 61–96

Murphy, J.B. & Sturm, E. (1925) Primary lung tumours in mice following the cutaneous application of coal tar. *J. exp. Med.*, **42**, 693–700

Nettesheim, P. (1972) Respiratory carcinogenesis studies with the Syrian golden hamster: A review. *Prog. exp. Tumour Res.*, **16**, 185–200

Nettesheim, P. & Hammons, A.S. (1971) Induction of squamous cell carcinoma in the respiratory tract of mice, *J. natl Cancer Inst.*, **47**, 697–701

Newberne, P.M. (1976) Experimental hepatocellular carcinogenesis. *Cancer Res.*, **36**, 1573–2578

Nogueira, E., Cardesa, A. & Mohr, U. (1993) Experimental models of renal carcinogenesis. *Cancer Res. clin. Oncol.*, **119**, 190–198

Ohmori, T., Hiasa, Y., Murata, T. & Williams, G.M. (1982) Gamma-glutamyl transpeptidase activity in carcinogen-induced epithelial lesions of rat kidney. *Gann*, **73**, 543–548

Okita, K., Noda, K., Fukumoto, Y. & Takemoto, T. (1976) Cytochrome P-450 in hyperplastic liver nodules induced by 2-acetylaminofluorene in rats. *Jpn. J. Cancer Res.*, **67**, 899–902

Pitot, H.C. (1990) Altered hepatic foci: Their role in murine hepatocarcinogenesis. *Ann. Rev. Pharmacol. Toxicol.*, **30**, 465–500

Pitot, H.C., Barsness, L., Goldsworthy, T. & Kitagawa, T. (1978) Biochemical characterization of stages of hepatocarcinogenesis after a single dose of diethylnitrosamine. *Nature*, **271**, 456–458

Pitot, H.C., Gross, L.E. & Goldsworthy, T. (1985) Genetics and epigenetics of neoplasia: facts and theories. *Carcinogenesis*, **10**, 65–79

Pour, P.M. & Hauser, R. (1989) Histogenesis of pseudoductular formation during pancreatic carcinogenesis in hamsters: Electron microscope and immunohistochemical studies. In: Schuller, H.M. ed., *Comparative Ultrastructural Pathology of Selected Tumours in Man and Animals*, Boca Raton, FL, CRC Press, pp. 43–60

Pour, P., Krueger, F.W., Althoff, J., Cardesa, A. & Mohr, U. (1974) Cancer of the pancreas induced in the Syrian golden hamster. *Am. J. Pathol.*, **76**, 349–358

Pozharisski, K.M., Likhachev, A.J., Klimashevski, V.F. & Shaposhnikov, J.D. (1979) Experimental intestinal cancer research with special reference to human pathology. *Adv. Cancer Res.*, **30**, 165–237

Pretlow, T.P., O'Riordan, M.A., Kolman, M.F. & Jurcisek, J.A. (1990) Colonic aberrant crypts in azoxymethane-treated F344 rats have decreased hexosaminidase activity. *Am. J. Pathol.*, **136**, 13–16

Pretlow, T.P., Oriordan, M.A., Somich, G.A., Amini, S.B. & Pretlow, T.G. (1992) Aberrant crypts correlate with tumour incidence in F344 rats treated with azoxymethane and phytate. *Carcinogenesis*, **13**, 1509–1512

Pylev, L.N. (1961) Experimental induction of lung cancer in rats by intratracheal introduction of 9,10-dimethyl-1,2-benzanthracene. *Bull. exp. Biol. Med.*, **52**, 99–102

Rabes, H.M. (1989) Cell proliferation and clonal development in hepatocarcinogenesis. In: Bannasch, P., Keppler, D. & Weber, G., eds, *Liver Cell Carcinoma*, London, Kluwer Academic Publishers, pp. 305–313

Rao, M.S., Upton, M.P., Subbarao, V. & Scarpelli, D.G. (1982) Two populations of cells with differing proliferative capacities in atypical acinar cell foci induced by 4-hydroxyaminoquinoline-1-oxide in the rat pancreas. *Lab. Invest.*, **46**, 527–534

Reddy, J.K., Svoboda, D.J. & Roa, M.S. (1974) Susceptibility of an inbred strain of guinea pigs to the induction of pancreatic adenocarcinoma by *N*-methyl-*n*-nitrosourea. *J. natl Cancer Inst.*, **52**, 991–993

Rehm, S. & Ward, J.M. (1989) Quantitative analysis of alveolar type II cell tumours in mice by whole lung serial and step sections. *Toxicol. Pathol.*, **17**, 737–742

Remandet, B., Gouy, D., Berthe, J., Mazue, G. & Williams, G.M. (1984) Lack of initiating or promoting activity of six benzodiazepine tranquilizers in rat liver limited bioassays monitored by histopathology and assay of liver and plasma enzymes. *Fundam. appl. Toxicol.*, **4**, 152–163

Reuber, M.D. (1965) Development of preneoplastic and neoplastic lesions of the liver in male rats given 0.025 percent *N*-2-fluorenyldiacetamide. *J. natl Cancer Inst.*, **34**, 697–723

Reuber, M.D. (1974) Histogenesis of preneoplastic and neoplastic lesions of the kidney in rats ingesting *N*-4-(4′-fluorobiphenyl)acetamide. *Pathol. Microbiol.*, **41**, 266–275

Reuber, M.D. (1975) Evolution of hyperplasia, hyperplastic nodules, and carcinomas of the liver induced in rats by *N*-2-fluorenyldiacetamide. *Gann Monogr. Cancer Res.*, **17**, 301–342

Reznik-Schüller, H. (1976) Ultrastructural alterations of nonciliated cells after nitrosamine treatment and their significance for pulmonary carcinogenesis. *Am. J. Pathol.*, **85**, 549–554

Rice, J.M. (1973) The biological behaviour of transplacentally induced tumours in mice. In: Tomatis, L., Mohr, U. & Davis, W., eds, *Transplacental Carcinogenesis* (IARC Scientific Publications No. 4), Lyon, IARC, pp. 71–83

Roebuck, B.D., Baumgartner, K.J. & Thron, C.D. (1984) Characterization of two populations of pancreatic atypical acinar cell foci induced by azaserine in the rat. *Lab. Invest.*, **50**, 141–146

Ruebner, B.H., Michas, C., Kanayama, R. & Bannasch, P. (1976) Sequential hepatic histologic and histochemical changes produced by diethylnitrosamine in the rhesus monkey. *J. natl Cancer Inst.*, **57**, 1261–1267

Russo, J. & Russo, I.H. (1987) Biology of disease biological and molecular bases of mammary carcinogenesis. *Lab. Invest.*, **57**, 112–137

Russo, I.H. & Russo, J. (1996) Mammary gland neoplasia in long-term rodent studies. *Environ. Health Perspectives*, **9**, 938–967

Saffiotti, U., Cefis, F. & Kolb, L. (1968) A method for the experimental induction of bronchogenic carcinoma. *Cancer Res.*, **28**, 104–124

Sargent, L., Xu, Y-H., Sattler, G.L., Meisner, L. & Pitot, H.C. (1989) Ploidy and karyotype of hepatocytes isolated from enzyme-altered foci in two different protocols of multistage hepatocarcinogenesis in the rat. *Carcinogenesis*, **10**, 387–391

Sasaki, T. & Yoshida, T (1935) Experimental induction of liver carcinoma by feeding o-aminoazotoluene. *Virchow's Arch. Pathol. Anat.*, **295**, 175–200 (in German)

Sato, K., Kitahara, A., Satoh, K., Ishikawa, T., Tatematsu, M. & Ito, N. (1984) The placental form of glutathione *S*-transferase as a new marker protein for preneoplasia in rat chemical hepatocarcinogenesis. *Gann*, **75**, 199–202

Schauer, A. & Kunze, E (1968) Enzyme histochemical and radioautographic studies during cancerization of the rat liver with diethylnitrosamine. *Z. Krebsforsch.*, **70**, 252–266

Scherer, E. & Emmelot, P. (1976) Kinetics of induction and growth of enzyme-deficient islands involved in hepatocarcinogenesis. *Cancer Res.*, **36**, 2544–2554

Schreiber, H., Schreiber, K. & Martin, D.H. (1975) Experimental tumour induction in a circumscribed region of the hamster trachea: Correlation of histology and exfoliative cytology. *J. natl Cancer Inst.*, **54**, 187–191

Schüller, H.M. (1987) Experimental carcinogenesis in the peripheral lung. In: McDowell, E.M., ed., *Lung carcinomas. (Current Problems in Tumour pathology*, Vol. 3), Edinburgh, Churchill Livingstone, pp. 243–254

Schüller, H.M. (1989) Comparative ultrastructural pathology of lung tumours. In: Schuller, H.M., ed., *Comparative Ultrastructural Pathology of Selected Tumours in Man and Animals*, Boca Raton, FL, CRC Press, pp. 2–39

Schulte-Hermann, R., Bursch, W. & Kraupp-Grasl, B. (1995) Active cell dealth (apoptosis) in liver biology and disease. In: Boyer, J.L. & Ockner, R.K., eds, *Progress in Liver Disease*, Vol. XIII, Philadelphia, W.B. Saunders Company, pp. 1–35

Sell, S. & Leffert, H.L. (1982) An evaluation of cellular lineages in the pathogenesis of experimental hepatocellular carcinoma. *Hepatology*, **2**, 77–86

Shabad, L.M. (1962) Experimental cancer of the lungs. *J. natl Cancer Inst.*, **28**, 1305–1332

Shay, H., Aegerter, E.A., Gruenstein, M. & Komarov, S.A. (1949) Development of adenocarcinoma of breast in Wistar rat following gastric instillation of methylcholanthrene. *J. natl Cancer Inst.*, **10**, 255–266

Shimkin, M.B. & Stoner, G.D. (1975) Lung tumours in mice: Application to carcinogenesis bioassay. *Adv. Cancer Res.*, **21**, 1–58

Shinozuka, H., Popp, J.A. & Konishi, Y. (1976) Ultrastructures of atypical acinar cell nodules in rat pancreas induced by 4-hydroxyaminoquinoline-1-oxide. *Lab. Invest*, **34**, 501–509

Shubik, P. (1950) The growth potentialities of induced skin tumours in mice. The effect of different methods of chemical carcinogenesis. *Cancer Res.*, **10**, 713–717

Sinha, D. & Dao, T.L. (1975) Site of origin of mammary tumours induced by 7,12-dimethylbenz[*a*]-anthracene in the rat. *J. natl Cancer Inst.*, **54**, 1007–1009

Slaga, T.J. & Nesnow, S. (1985) SENCAR mouse skin tumourigenesis. In: Milman, H.A. & Weisburger, E.K., eds, *Handbook of Carcinogen Testing*, Park Ridge, NJ, Noyes Publications, pp. 230–250

Slaga, T.J., Fischer, S.M., Triplett, L.L. & Nesnow, S. (1981) Comparison of complete carcinogenesis and tumour initiation and promotion in mouse skin: The induction of papillomas by tumour initiation–promotion, a reliable short term assay. *J. environ. Pathol. Toxicol.*, **4**, 1025–1041

Squartini, F. (1979) Tumours of the mammary gland. In Turusov, V.S., ed., *Pathology of Tumours in Laboratory Animals*, Volume II, *Tumours of the Mouse* (IARC Scientific Publications No. 23), Lyon, IARC, pp. 43–90

Steinmetz, K.L. & Klaunig, J.E. (1996) Transforming growth factor-I in carcinogen-induced F344 rat hepatic foci. *Toxicol. appl. Pharmacol.*, **140**, 131–145

Stenbäck, F. (1977) Morphology of experimentally induced respiratory tumours in Syrian Golden hamster. A histological and ultrastructural study. *Acta otolaryngol.*, **347**, 1–59

Stenbäck, F. (1978a) Life history and histopathology of ultraviolet light-induced skin tumours. *Natl Cancer Inst. Monogr.*, **50**, 57–70

Stenbäck, F. (1978b) Tumour persistence and regression in skin carcinogenesis. *Z. Krebsforsch.*, **91**, 249–259

Stenbäck, F., Mori, H., Furuya, K. & Williams, G.M. (1986) Pathogenesis of dimethylnitrosamine-induced hepatocellular cancer in hamster liver and lack of enhancement by phenobarbital. *J. natl Cancer Inst.*, **76**, 327–333

Stenbäck, F., Gebhardt, R., Sirma, H., Garbay, J.-M. & Williams, G.M. (1994) Sequential functional and morphological alterations during hepatocarcinogenesis induced in rats by feeding of a low dose of 2-acetylaminofluorene. *Toxicol. Pathol.*, **22**, 620–632

Stewart, H.L., Dunn, T.B., Snell, K.C. & Deringer, M.K. (1979) Tumours of the respiratory tract. In: Turusov, V.S., ed., *Pathology of Tumours in Laboratory Animals*, Volume II, *Tumours of the Mouse* (IARC Scientific Publications No. 23), Lyon, IARC, pp. 251–287

Stewart, H.L., Williams, G., Keysser, C.H., Lombard, L.S. & Montali, R.J. (1980) Histologic typing of liver tumours of the rat. *J. natl Cancer Inst.*, **64**, 177–206

Stoner, G.D. & Shimkin, M.B. (1985) Lung tumours in strain A mice as a bioassay for carcinogenicity. In: Milman, H.A. & Weisburger, E.K., eds, *Handbook of Carcinogen Testing*, Park Ridge, NJ, Noyes Publications, pp. 179–214

Stopera, S.A., Murphy, L.C. & Bird, R.P. (1992) Evidence for a *ras* gene mutation in azoxymethane-induced colonic aberrant crypts in Sprague-Dawley rats: earliest recognizable precursor lesions of experimental colon cancer. *Carcinogenesis*, **13**, 2081–2085

Sudilovsky, O. & Hei, T.K. (1991) Aneuploidy and progression in promoted preneoplastic foci during chemical hepatocarcinogenesis in the rat. *Cancer Lett.*, **56**, 131–135

Takahashi, M., Arai, H., Kokubo, T., Furukawa, F., Kurata, Y. & Ito, N. (1980) An ultrastructural study of precancerous and cancerous lesions of the pancreas

in Syrian golden hamsters induced by *N*-nitroso-bis(2-oxopropyl) amine. *Gann*, **71**, 825–831

Tanaka, T., Mori, H., Takahashi, M. & Williams, G.M. (1984) DNA content of hyperplastic and neoplastic acinar cell lesions in rat and human pancreas. *J. exp. Pathol.*, **1**, 315–326

Thorgeirsson, S.S. & Evarts, R.P. (1992) Growth and differentiation of stem cells in adult rat liver. In: Sirica, A.E., ed., *The Role of Cell Types in Hepatocarcinogenesis*, Boca Raton, FL, CRC Press, pp. 109–120

Timme, A.H. (1978) Hyperplastic foci in precancerous rat liver: Light microscopic and electron microscopic study. *J. natl Cancer Inst.*, **61**, 407–409

Tsuda, H., Hasegawa, R., Imaida, K., Masui, T., Moore, M.A. & Ito, N. (1984a) Modifying potential of thirty-one chemicals on the short-term development of gamma-glutamyltranspeptidase positive foci in diethylnitrosamine-initiated rat liver. *Gann*, **75**, 876–883

Tsuda, H., Sakata, T. Masui, T., Imaida, K. & Ito, N. (1984b) Modifying effects of butylated hydroxyanisole, ethyoxyquin and acetaminophen on induction of neoplastic lesions in rat liver and kidney initiated by *N*-ethyl-*N*-hydroxyethylnitrosamine. *Carcinogenesis*, **5**, 525–531

Vesselinovitch, S.D., Hacker, H.J. & Bannasch, P. (1985) Histochemical characterization of focal hepatic lesions induced by single diethylnitrosamine treatment in infant mice. *Cancer Res.*, **45**, 2774–2780

Vivona, A.A., Shpitz, B., Medline, A., Bruce, WR., Hay, K., Ward, M.A., Stern, H.S. & Gallinger, S. (1993) K-*ras* mutations in aberrant crypt foci, adenomas and adenocarcinomas during azoxymethane-induced colon carcinogenesis. *Carcinogenesis*, **14**, 1777–1781

Walpole, A.L., Williams, M.H.C. & Roberts, D.C. (1952) The carcinogenic action of 4-aminodiphenyl and 3:2′-dimethyl-4-aminodiphenyl. *Br. J. ind. Med.*, **9**, 255–263

Wargovich, M.J., Chen, C.-D., Jimenez, A., Steele, V.E., Velasco, M., Stephens, L.C., Price, R., Gray, K. & Kelloff, G.J. (1996) Aberrant crypts as a biomarker for colon cancer: Evaluation of potential chemoprevention agents in the rat. *Cancer Epidemiol. Biomarkers Prev.*, **5**, 355–360

Watanabe, K. & Williams, G.M. (1978) Enhancement of rat hepatocellular-altered foci by the liver tumour promoter phenobarbital: evidence that foci are precursors of neoplasms and that the promoter acts on carcinogen-induced lesions. *J. natl Cancer Inst.*, **61**, 1311–1314

Weisburger, J.H. & Fiala, E.S. (1983) Experimental colon carcinogens and their mode of action. In: Autrup, H. & Williams, G.M., eds, *Experimental Colon Carcinogenesis*, Boca Raton, FL, CRC Press, pp. 27–50

Weisburger, J.H. & Williams, G.M. (1984) Bioassay of carcinogens: *in vitro* and *in vivo* tests. In: Searle, C.E., ed., *Chemical Carcinogens*, Vol. 2 (ACS Monograph 182), 2nd Ed., Washington, DC, American Chemical Society, pp. 1323–1373

Weisburger, J.H., Rivenson, A., Hard, G.C., Zang, E., Nagao, M. & Sugimura, T. (1994) Role of fat and calcium in cancer causation by food mutagens, heterocyclic amines. *Proc. Soc. exp. Biol. Med.*, **205**, 347–352

Welsch, C.W. (1985) Host factors affect the growth of carcinogen-induced rat mammary carcinomas: A review and tribute to Charles Brenton Huggins. *Cancer Res.*, **45**, 3415–3445

Williams, G.M. (1976) Functional markers and growth behavior of preneoplastic hepatocytes. *Cancer Res.*, **36**, 2540–2543

Williams, G.M. (1980) The pathogenesis of rat liver cancer caused by chemical carcinogens. *Biochim. biophys. Acta*, **605**, 167–189

Williams, G.M. (1982) Phenotypic properties of preneoplastic rat liver lesions and applications to detection of carcinogens and tumour promoters. *Toxicol. Pathol.*, **10**, 3–10

Williams, G.M. (1989) The significance of chemically-induced hepatocellular altered foci in rat liver and application to carcinogen detection. *Toxicol. Pathol.*, **17**, 663–674

Williams, G.M. & Watanabe, K. (1978) Quantitative kinetics of development of *N*-2-fluorenylacetamide-induced, altered (hyperplastic) hepatocellular foci resistant to iron accumulation and of their reversion or persistence following removal of carcinogen. *J. natl Cancer Inst.*, **61**, 113–121

Williams, G.M. & Yamamoto, R.S. (1972) Absence of stainable iron from preneoplastic and neoplastic lesions in rat liver with 8-hydroxyquinoline-induced siderosis. *J. natl Cancer Inst.*, **49**, 685–694

Williams, G.M., Klaiber, M., Parker, S.E. & Farber, E. (1976) Nature of early appearing, carcinogen-induced liver lesions resistant to iron accumulation. *J. natl Cancer Inst.*, **57**, 157–165

Witschi, H. & Haschek, W.M. (1983) Cells of origin of lung tumours in mice. *J. natl Cancer Inst.*, **70**, 991–992

Yamagiwa, K. & Ichikawa, K. (1915) Experimental studies on the pathogenesis of epithelial tumours. *Mitt. Med. Facultät Kaiserl. Univ. Tokyo*, **15**, 295–344 (in German)

Yamaguchi, S., Hakoi, K., Ozaki, K., Kato, T., Tiwawech, D., Nagao, S., Takahashi, H., Matsumoto, K. & Tsuda, H. (1993) Number of simultaneously expressed enzyme alterations correlates with progression of *N*-ethyl-*N*-hydroxyethylnitrosamine-induced hepatocarcinogenesis in rats. *Jpn. J. Cancer Res.*, **84**, 1237–1244

Yuspa, S.H. (1994) The pathogenesis of squamous cell cancer: Lesions from studies of skin carcinogenesis. Thirty-third G.H.A. Clowes Memorial Award Lecture. *Cancer Res.*, **54**, 1178–1189

Zerban, H., Radig, S., Kopp-Schneider, A. & Bannasch, P. (1994) Cell proliferation and cell death (apoptosis) in hepatic preneoplasia and neoplasia are closely related to phenotypic cellular diversity and instability. *Carcinogenesis*, **15**, 2467–2473

Zhang, X.M., Stamp, D., Minkin, S., Medline, A., Corpet, D.E., Bruce, W.R. & Archer, M.C. (1992) Promotion of aberrant crypt foci and cancer in rat colon by thermolyzed protein. *J. natl Cancer Inst.*, **84**, 1026–1030

Corresponding author:

G.M. Williams
American Health Foundation, 1 Dana Road, Valhalla, NY 10595, USA