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ROLE OF PERSISTENT, NON-GENOTOXIC TISSUE DAMAGE IN RODENT CANCER AND RELEVANCE TO HUMANS

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INTRODUCTION

Historical Background

Experimental induction of cancer by chemicals was first reported in detail in 1918 when repeated application of coal tar to the ear of rabbits resulted in skin carcinomas (1). Over the next few years, other workers confirmed this finding and demonstrated similar effects in mice and rabbits from the application of soot extracts, other types of tar (e.g. acetylene or isoprene), and some heated mineral oils (2, 3). These early workers also observed skin "irritation", sometimes accompanied by ulcers, at the site of application of the test material. "Irritation" was thought to be an important factor in skin tumor development.

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However, not all irritants (e.g. acridine) induced skin cancer in mice (2, 4) and, conversely, some purified chemicals isolated from these crude materials produced a high incidence of skin tumors with little or no "irritation" (5, 6). These observations suggested that intracellular changes induced by carcinogens may lead to cancer, independent of any lesions observable visually or by light microscopy. The hypothesis was thus proposed that cancer results from the interaction of a chemical with a "sensitive" site in the cell, conferring on it the property of unrestrained proliferation.

Genotoxic and Nongenotoxic Carcinogens

This hypothesis has gained considerable support in recent years from the demonstration that many chemical carcinogens are capable of producing radicals that bind with various cell constituents, including DNA. Interaction with DNA is of primary importance since this may lead to a mutagenic event that is now considered an essential step in the carcinogenic process. Such carcinogens with demonstrable mutagenic activity are termed generically *genotoxic* carcinogens (7, 8).

However, various chemicals that are not genotoxic are also capable of inducing tumors in experimental animals (9). These so-called *nongenotoxic* carcinogens usually induce cancer when administered throughout the animal's lifespan at high doses. At such levels, marked biochemical and pathological changes invariably occur, shortly after treatment commences, in the organ in which cancer eventually appears.

Pathological and Adaptive Cellular Changes

This review focuses on the major biochemical and pathological changes that take place in the early and intermediate stages of treatment with nongenotoxic chemicals that induce cancer in long-term rodent studies and also examines the correlation between these changes and the development of tumors. Attention is directed particularly to changes that persist from the time they first appear to the time tumors eventually develop.

Changes such as fatty change, cell necrosis, or hydropic degeneration are readily accepted as pathological in nature and hence as an expression of organ damage. Other, more subtle changes (e.g. organ enlargement or increases in enzyme activity or the size and number of cellular organelles) are less readily acknowledged as being indicative of damage. Although such changes are outside the "normal" range, there would be a tendency to regard them as "adaptive" phenomena rather than as signs of injury.

Adaptation in response to the administration of a chemical implies that to meet an increased metabolic demand, a call is made on the biological reserves of an organ. This demand is proportional to the dose administered and the duration of treatment. With low doses or short treatment periods the adaptive change could readily be handled within the capacity of the homeostatic

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control mechanism such that on withdrawal of treatment no residual effect would persist. At high doses given over prolonged periods the demand may be close to or even exceed the limits of homeostasis. In such a circumstance, any breakdown in homeostatic control that occurs could lead to overt pathological changes; such adaptive changes would be regarded as precursors of organ damage. Furthermore, the capacity to maintain adaptive changes decreases with age so that prolonged administration of a chemical may eventually produce a breakdown in homeostatic mechanisms of an ageing animal even at doses that are well tolerated in the young animal (10, 11).

In this review both the pathological and adaptive changes have been considered and to justify a causal association between these changes and eventual neoplasia in experimental animals three types of evidence have been sought: (*a*) demonstration of a threshold dose below which both the early/intermediate changes and the neoplastic changes are absent but above which both types of change are manifest; (*b*) regression of the early/intermediate changes and failure of tumors to appear when treatment is prematurely stopped and is followed by an adequate treatment-free observation period; and (*c*) suppression of both the early/intermediate and the neoplastic changes by an inhibitory agent.

Although the evidence obtained from various studies on different organs/tissues may range in degree from tenuous to adequate, the ensuing sections show that, taken overall and regardless of site, there is compelling evidence that persistent damage, if sustained by prolonged exposure to nongenotoxic carcinogens, is a major contributory factor in tumor development.

CONNECTIVE TISSUE DAMAGE AND SUBCUTANEOUS SARCOMA IN RODENTS

One of the first indications that factors, other than chemical interaction with specific intracellular sites, could be responsible for tumor induction was the discovery in the 1940s of sarcomas around solid materials implanted into rats and mice. Thus, fibrosarcomas were observed around large bakelite discs implanted subcutaneously in rats (12). Oppenheimer's interest in the treatment of hypertension led him to develop a "hypertensive" rat model by wrapping pieces of cellophane sheet around the kidneys to produce partial cortical ischemia (13). A large tumor developed around the cellophane film in some of the rats after several months. To investigate this unexpected finding, pieces of cellophane films were implanted into the subcutaneous tissue of the rat and tumors also developed around these implants. The tumors were, histologically, sarcomas. No tumors developed when the cellophane was implanted subcutaneously in the shredded form (14–16).

The finding of "solid-state" carcinogenesis attracted much attention be-

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cause it appeared when theories on the importance of molecular interaction in carcinogenesis were in vogue. Many experiments confirmed the Oppenheimer finding and indeed, for a time, the induction of sarcomas by solid implants became known as the "Oppenheimer effect". All solids (including noble metals such as gold, silver, or platinum) tested by implantation induced sarcomas and the shape and particularly the size of the implant had a determining influence on sarcoma induction (17). In general, solid pieces measuring 2 cm in diameter or 2 cm square, when implanted subcutaneously into rats and mice, induced a high incidence of tumors. Smaller pieces induced fewer tumors, while the material in shredded form was inert, even though the total weight of its fragments was the same as that of the unshredded piece. This finding challenged the molecular theory of carcinogenesis since the area of interaction between the surface molecules of fragments and cell components was much greater than that with solid pieces. This prompted a study of the tissue reaction in an attempt to explain the mechanism of tumor induction.

A foreign body implanted in any mammalian organ or tissue induces a foreign-body reaction with the eventual formation of a fibrous capsule. In the subcutaneous tissue of rats, large foreign bodies produced a thick fibrous reaction that persisted and from which sarcoma developed. The importance of the fibrous reaction in sarcoma induction was evident from the observation that removal of a large foreign body after 5 or 6 months led to the development of a thick connective tissue reaction that did not resolve and tumors developed. However, removal of the foreign body before the fourth month led to complete regression of the capsule and no tumors developed (15, 17–21). Implanted foreign bodies, sufficiently small in size not to induce sarcoma, produced only a mild fibrous reaction (17). These studies confirmed that the mechanism of sarcoma induction was in some way linked with the continued presence of the fibrous tissue reaction.

The question as to the validity of subcutaneous sarcoma induction as an index of carcinogenicity arose again when iron-dextran was found to produce this type of tumor following repeated subcutaneous injections in rats or mice (22). The tumors were preceded by the formation of a thick granulomatous reaction rich in iron-containing macrophages (23).

The predictive value of subcutaneous sarcoma development as an index of carcinogenicity was further scrutinized when the question arose in the early 1960s whether sarcoma induction by food colorings in rodents was indicative of a human carcinogenic risk. Repeated subcutaneous injections of hypertonic solutions of glucose (25% w/v in water) or salt (4% w/v in water) for several months were known to produce local sarcomas in rats and mice (24–26). However, this finding was dismissed as trivial and was presumed to be due to osmotic shock (27). This explanation spared regulatory authorities from having to pronounce glucose, xylose, and other monosaccharides as car-

Table 1 Relationship between physicochemical properties of various food colorings and subcutaneous sarcoma development in rats (28–30)

Food color	Surface activity	Amphipathy	Sarcoma
Blue VRS	+		+
Brilliant Blue FCF	+		+
Patent Blue V Na	+		+
Patent Blue V Ca	−	−	−
Amaranth	−	−	−
Eosine G		+	+
Rhodamine B		+	+

+ = present − = absent

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cinogenic. But this relaxed attitude did not extend to food colorings because they induced sarcomas when administered at much lower concentrations than sugars. There were, however, some important anomalies—two colorings structurally similar to those that gave rise to sarcomas produced no tumors when repeatedly injected subcutaneously as aqueous solutions into rats (Table 1; 28, 29). Subsequent investigations revealed that the sarcoma-inducing food colorings possessed either surface-active or amphipathic properties when dissolved in water at concentrations that produced sarcomas. Furthermore, severe tissue damage was produced when such concentrations were injected subcutaneously (30).

The importance of these physical properties and of the attendant tissue damage became further evident from a study in which a surface-active coloring (Patent Blue V) was repeatedly injected subcutaneously at four different concentrations, such that adjustment of the dose volume maintained a constant dose by weight in all groups of rats (Table 2). When the coloring was administered at surface-active concentrations high enough to produce massive fibrosis, sarcomas developed, but neither effect was seen at concentrations below the surface-active concentration (30).

The principal mechanism believed to be involved in sarcoma induction is the continued activation of the reparative process in connective tissues by the physical, tissue-damaging property of the coloring.

LIVER CHANGES AND TUMORS IN RODENTS

Compounds Causing Histological Damage

The importance of hepatic damage in the development of liver carcinoma has long been suspected, as patients with cirrhosis of the liver were found to be at greater risk of developing hepatocellular carcinoma than those without cirrhosis (31).

Table 2 Relationship between surface tension, type of tissue reaction and sarcoma production in rats treated with various concentrations of Patent Blue V sodium (30)

Concentration in water (%)	Dose (ml)	Surface tension reduction (%)	Tissue reaction type	Sarcoma incidence (%)
0	1.5	0	Mild	0
1.0	1.5	15	Mild	0
2.0	1.0	50	S/P	56
3.0	0.5	50	S/P	61

S/P = Severe/progressive

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In 1942 (32), it was shown that a total of 53 oral doses of CCl_4 given 2–3 times weekly to mice produced extensive centrilobular necrosis, leading to chronic lesions in which the regenerated parenchyma was divided by connective tissue strands into discrete nodules, called "hepatomas". These nodules were transplantable in homozygous mice and were therefore thought to possess the property of autonomous growth. If treatment was discontinued before 23 oral doses were administered, no such lesions occurred, suggesting the existence of a "time threshold" (32).

In another study (33), three large oral doses of CCl_4, given within 2–3 weeks of each other, resulted in extensive hepatocellular necrosis in mice but no hepatomas developed within a 6-month posttreatment observation period. When the same total dose was given as a large number of smaller oral hepatotoxic doses, 2–3 days apart for approximately 2 months, both necrosis and hepatomas developed in the 6-month posttreatment observation period. These findings were interpreted as indicating that the production of cycles of necrosis and regeneration, repeated at short intervals and maintained over a prolonged period, is causally related to the production of tumors in this experimental model (33).

A similar phenomenon occurs in rats. Repeated subcutaneous administration of CCl_4 produced cirrhosis and neoplasia in the liver (34). Since CCl_4 is not genotoxic (35, 36), it seems likely that the chronic tissue injury played an important role in the development of hepatic tumors.

In further investigations (37), mice were given various doses of $CHCl_3$, which is also nongenotoxic (38, 39), and hepatomas developed only at oral doses high enough to produce hepatocellular necrosis. All hepatoma-bearing mice also exhibited cirrhosis. It was concluded that the cycle of necrosis and regeneration produced by each successive dose was responsible for the production of hepatomas.

These studies support the view that sustained hepatocellular damage in

rodents induced by nongenotoxic agents, such as CCl_4 and $CHCl_3$, predisposes to liver tumor development.

Other examples of nongenotoxic compounds that are hepatotoxic and hepatocarcinogenic in rats or mice include tetrachloroethylene, 1,1,2,2–tetrachloroethane, and paracetamol (40).

Compounds Causing Subcellular Changes

Liver damage at the subcellular level may manifest itself as a disturbance in any cell organelle (41–43). Most information on the relationship between subcellular damage and tumor production concerns lysosomes and mitochondria.

Disturbance of the lysosomal pattern was the earliest manifestation of hepatocellular injury in an 85-week rat feeding study in which the food coloring Ponceau MX (a nongenotoxic agent) was given at a dietary level of 2%. Autophagic vacuoles, derived from enlarged lysosomes, appeared after 8 weeks and their appearance coincided with a return of elevated mixed-function oxidase (MFO) activity to the level seen in controls, even though the compound continued to be administered. Prior to week 8, MFO activity was about 6 times the normal level. The disappearance of this "adaptive" change was presumed to have triggered the cytological damage that manifested itself as a pertubation of the lysosomal system. Several weeks later extensive fatty change and nodular hyperplasia were found in the treated rats and these changes were followed by the appearance of well-differentiated trabecular carcinomas that metastasized to the lung (44, 45).

These sequential changes indicate that the appearance of tumors followed a progressively intensifying hepatocellular damage first evident at the subcellular level. A clear indication of the role played by liver damage in tumor production came from a dose-response experiment on Ponceau MX. A high incidence of liver tumors occurred in animals given the coloring at dietary levels known to cause lysosomal disturbances, whereas no tumors were obtained at lower levels where the hepatotoxic effect was absent or equivocal (11, 46).

Mitochondrial damage also appears to be a determining factor in the neoplastic process and is thought to be involved in the production of tumors by methapyrilene. This nongenotoxic agent is hepatocarcinogenic to the rat (47) and early studies revealed mitochondrial proliferation that reversed to normality on cessation of treatment (48).

The effect of subcellular injury on the turnover rate of liver cells appears not to have been investigated in depth. It is, however, likely that chronic subcellular injury would impair cell survival, leading to an increased production of liver cells to replace those that are lost. Thus, following subcellular injury, hyperplasia is very probably a feature of the hepatic reparative re-

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Table 3 Examples of nongenotoxic chemicals producing enzyme induction, enlargement and tumors in the liver of rats and mice (40)

Chemical	MFO induction	Liver enlargement	Liver tumors
Chlordecone	+	++	++
Phenobarbitone	++	++	+
Polychlorinated biphenyls	++	++	+
Butylated hydroxytoluene	+	++	+
DDT	++	++	+
Dieldrin	+	++	+
α-Hexachlorocyclohexane	++	++	++
Chlordane	++	++	+

+ = Mild ++ = Marked

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sponse (despite the absence of overt cell necrosis) and this may be responsible for tumor production in a way similar to that observed after overt cell necrosis.

Compounds Causing Liver Enlargement (Without Initial Liver Damage)

Various nongenotoxic compounds that initially do not induce histological damage to the liver even at moderately high doses may still induce hepatocellular carcinoma in rodents after life-time administration of high doses. Some of these compounds are shown in Tables 3 (40) and 4 (49) and further examples are cited elsewhere (11). Although cells in enlarged livers often show biochemical and ultrastructural changes, there is no clear indication that these changes are indicative of cell damage. On the contrary, such changes are widely regarded as an "adaptive" phenomenon. The biochemical changes mostly studied relate to (*a*) the cytochrome P-450 system of MFO enzymes and (*b*) the peroxisome and its associated enzymes.

It is not intended to review these biochemical changes in detail but only to draw attention to the relationship between them and liver enlargement on the one hand and the association of these two phenomena with the eventual development of hepatocellular carcinoma on the other.

INDUCERS OF THE MFO SYSTEM A considerable increase in the activity of cytochrome P-450 and of a variety of its associated enzymes (principally MFO) has been reported in the liver in many biochemical investigations on compounds that produce liver growth (Table 3; 11, 50). Ultrastructural studies of the hepatocytes also showed a considerable increase in the smooth endoplasmic reticulum (SER), the organelle containing most of these enzymes (11, 50).

Table 4 Examples of peroxisome proliferators that induce hepatomegaly and liver cancer in rats and mice (49)

Compound	Species
Clofibrate	Rat
Wy-14,643	Mouse
Nafenopin	Rat, mouse
Tibric acid	Rat
BR-931	Rat, mouse
Di(2-ethylhexyl)phthalate	Rat, mouse
Di(2-ethylhexyl)adipate	Rat, mouse
Fenofibrate	Rat, mouse
Ciprofibrate	Rat, mouse

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These changes occur during the first 3–4 days of the administration of the enzyme-inducing compound. Detailed time-course studies show the earliest effects to be increases in MFO activity and in DNA synthesis in the hepatic nuclei within 24 hours, followed by a wave of mitotic activity after 1–3 days. The changes are accompanied by a progressive increase in liver weight, which reaches a maximum by 4–6 days in the rat and longer in the mouse. The increases in liver weight and MFO activity are dose-related (11, 51–53).

In most studies, liver mass increased by 10 to 50% but increases of 100% or more have been reported, particularly in mice, with large doses of enzyme-inducing compounds (11).

Surprisingly, compounds that induce liver growth may not necessarily induce MFO activity and, conversely, enzyme activity may be induced without an increase in liver weight. Thus, while phenobarbitone, butylated hydroxytoluene, hexachlorocyclohexane, and chlordane induce both enzyme activity and liver growth, no such correlation was observed in a comparative study of substituted phenols in rats. When administered to rats, 2,4-di-*tert*-butyl-6-methylphenol, 2,6-di-*tert*-butyl-4-methylphenol (BHT), and 2,6-di-*tert*-butyl-4-ethylphenol showed moderate to potent enzyme-inducing activity and produced marked liver enlargement; on the other hand, 2-*tert*-butyl-5-methylphenol and 2,6-di-*tert*-butyl-4-α-methoxymethylphenol produced marked liver enlargement without any enzyme induction and, conversely, 2-*tert*-butyl-4-methylphenol and 2,4-di-*tert*-butyl-5-methylphenol induced enzyme activity with little or no effect on liver weight (54). Similarly, α-hexachlorocyclohexane produced in rats a doubling of liver size but only a 30% increase in aminopyrene demethylase activity, whereas the γ-isomer induced almost an 100% increase in enzyme activity but only a 20% increase in liver weight (Table 5; 55)

All the chemicals shown in Table 3 induce both liver growth and MFO

Table 5 Association between liver enlargement, enzyme induction and liver cancer in rats treated with hexachlorocyclohexane isomers (55)

	Increase, as % of control value		
Isomer	Liver enlargement	Increase in aminopyrene demethylase activity	Carcinoma
β	40	30	−
δ	30	30	NT
α	100	30	+
γ	20	100	−

− = Absent + = Present NT = Not tested

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activity and also produce liver tumors in mice and rats. Many workers have regarded enzyme induction as more important than liver growth in the production of liver cancer. In our view, not enough compounds that induce liver growth with or without enzyme induction have been studied to arrive at this conclusion and evidence from the hexachlorocyclohexane isomers suggests that induction of liver growth may be at least as important as enzyme induction in determining the outcome of hepatic neoplasia (Table 5). Unfortunately, virtually nothing is known of the hepatic changes that take place between the initial period of enzyme induction/liver growth and the development of tumors.

INDUCERS OF PEROXISOME PROLIFERATION The peroxisome was discovered in 1956 (56) and appears under the electron microscope as a spherical or oval organelle about 0.5μ in diameter surrounded by a single membrane and containing a fairly uniform dense matrix. Most peroxisomes contain a central crystalline structure consisting of uricase. Peroxisomes contain a range of enzymes involved in the metabolism of fatty acids, two of which have served as markers of peroxisomal activity—palmitoyl-CoA oxidase and carnitine acetyl transferase. The peroxisome also contains catalase, which metabolizes the hydrogen peroxide formed on fatty acid oxidation to active oxygen and water; thus the organelle performs both catalytic and peroxidative functions (49, 57).

Various compounds (Table 4) increase the number of peroxisomes within liver cells. Morphometric studies have shown that when the numbers of peroxisomes are increased, the volume of the hepatocyte occupied by the peroxisomes (volume density) is significantly increased. In rats, this increase in volume density may range from 20–30% following administration of cholestyramine or nicotinic acid (58) to 500–700% after treatment with clofibrate or ciprofibrate (49, 57). Such high increases are invariably accompanied by a marked increase in the activity of certain "marker" enzymes.

Three principal phenomena are associated with the induction of peroxisomal activity: (*a*) hepatic growth; (*b*) lipofuscin formation; and (*c*) development of hepatocellular neoplasia.

Association with liver enlargement Compounds that induce peroxisomal proliferation invariably induce also hepatomegaly (Table 4). Studies with fenofibrate (59) and di(2-ethylhexyl)phthalate (DEHP) (60) have shown that both peroxisomal proliferation and liver growth are dose-related.

The development of liver growth following administration of peroxisomal proliferators shows some similarity to that produced by MFO inducers. With fenofibrate or DEHP the liver grows rapidly in the first 3–4 days of administration. The uptake of ^{3}H-thymidine peaks on the third day and then reverts to normal levels, but liver growth continues slowly for a week or two and morphometric studies link this additional increase to hepatocyte enlargement. The enlarged liver returns to normal 2–3 weeks after treatment ceases. With continued administration, the liver remains enlarged (59, 60).

Association with lipofuscin formation Lipofuscin, for many years regarded as an "age pigment", is now generally accepted as resulting from the polymerization of oxidized lipid within the lysosome (61). Large deposits of this pigment were found present in the liver lysosomes of rats and mice treated with various hypolipidemic compounds for several months (57). Sequential studies of the early changes produced in cell organelles showed that this pigment appears in the lysosome within 2–3 weeks of treatment with fenofibrate (59) or DEHP (60) and accumulates progressively until the end of the 2-year studies (62). The rate of deposition of the pigment appears to be dose- and time-related. This accounts for the large deposits observed (49, 57) at the end of carcinogenicity studies in which the compounds were administered at or close to the maximum tolerated dose. The formation of the pigment intracellularly provides strong presumptive evidence that intracellular peroxidative activity is taking place and this peroxidative activity is regarded as a mild toxic effect (61). According to Reddy (57), this peroxidative activity is due to the formation of excess H_2O_2 and could also account for tumor formation since H_2O_2 is a genotoxic agent. There is however little evidence that hepatocellular carcinoma induced by peroxisomal proliferators is due to the H_2O_2 generated (62, 63) and attempts to induce unscheduled DNA synthesis by peroxisome proliferators have been unsuccessful (64, 65).

Association with hepatocellular adenoma or carcinoma Many months elapse from the appearance of these early changes to the eventual appearance of tumors, but the changes occurring during this intermediate period have

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been little studied until recently, despite their potential value in contributing to our understanding of the mechanism of tumor production. During this intermediate period, Wy-14,643, a potent inducer of peroxisomes and of hepatocellular carcinomas, considerably increases the rate of ^{3}H-thymidine uptake by liver cells (indicative of cellular proliferation), but in view of the attainment of a steady liver weight it would appear that this is accompanied by an increased rate of apopotosis (natural cell loss) (62, 64). Lipofuscin accumulation, indicative of a mild toxic effect (61), also increases progressively (62). Thus, with Wy-14,643, toxic and proliferative phenomena are present in the enlarged liver during this intermediate phase.

Evidence of increased mitotic activity and lipofuscin accumulation in rat liver was also obtained during this intermediate period with fenofibrate administered at a dose known to induce hepatocellular carcinomas in long-term studies (59).

DEHP, a less potent liver carcinogen than either Wy–14,643 or fenofibrate, failed to increase mitotic activity or ^{3}H-thymidine uptake in rat liver in the intermediate period but lipofuscin accumulation was increased for a limited period before it stabilized (62).

Apart from lipofuscin deposition, a centrilobular loss of glycogen and glucose-6-phosphatase activity were also observed in the early stages of treatment with DEHP and fenofibrate (59, 60), suggesting that a mild degree of hepatotoxicity was present. As mentioned earlier, a mild degree of hepatotoxicity may well reduce the lifespan of the hepatocyte, leading to increased cell proliferation in the enlarged liver. Note that lipofuscin accumulation, liver enlargement, peroxisomal proliferation, and hepatocellular carcinoma are all dose-related (59–62, 65). Dose-response studies carried out with fenofibrate and DEHP indicate that, at levels below the threshold dose for the induction of hepatocellular carcinoma, there is only a modest induction of peroxisomal enzymes, no evidence of lipofuscin accumulation, and less than a 50% increase in liver weight (59–62).

DIETARY AND HORMONAL FACTORS Dietary proteins, like xenobiotics, induce liver growth when administered to rodents in significant amounts (11, 66, 67). A diet containing 9, 19, 27, 36, or 45% casein, together with corn starch and other essential dietary constituents fed to mice over their lifespan, resulted in hepatic tumor incidences of 10, 38, 43, 50, and 33%, respectively, in the various casein groups (68). The relatively low incidence with 45% casein was attributed to the unpalatable nature of the diet (68). These findings endorse the link between liver enlargement and the development of hepatic tumors. Neither the early nor the "intermediate" stage cellular changes in tumor development have been studied.

Mestranol and ethinylestradiol, used as estrogens in oral contraceptive

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Table 6 Absence of hepatocarcinogenicity at threshold doses for liver enlargement in rodents treated with nongenotoxic carcinogens

Compound	Threshold dose for liver enlargement[a]	Species	Reference
Phenobarbitone	30 mg/kg bw[b]	Mouse	11, 69a
α-Hexachlorocyclohexane	10 ppm[c]	Rat	11, 69a
DDT	128 ppm[c]	Rat	11, 69a
Dieldrin	1.0 ppm[c]	Rat	11, 69a
BHT	75 mg/kg bw[b]	Rat	11, 69a
DEHP	50 mg/kg bw[b]	Rat	60
Fenofibrate	13 mg/kg bw[b]	Rat	59

[a] No tumors of the liver have been reported in long-term studies at these or lower doses
[b] Daily oral doses given
[c] Dietary concentration

products, each induced hepatic tumors in a dose-related manner in rats when administered alone or in combination with other steroids at doses that are equivalent to 2 to 400 times the human dose (69). Estrogens are known to induce liver enlargement in the rat (11).

Association Between Liver Enlargement and Tumor Induction

Liver enlargement induced by compounds (MFO inducers and peroxisome proliferators), as discussed earlier, has three characteristics: (*a*) onset within 2–3 days of start of treatment (11); (*b*) association with a wave of mitoses between 18–36 hours of start of treatment (11); and (*c*) persistence of liver enlargement until appearance of tumors (59, 60).

At dose levels where liver enlargment is absent or minimal, tumors do not develop (Table 6), again underlining the significance of marked liver enlargement as a factor in the development of tumors by nongenotoxic agents.

The early hyperplastic episode appears to be primarily responsible for the liver growth that occurs within the first 2–3 days of treatment.

Despite the link between liver enlargement and hepatic neoplasia, little is known about the hepatic changes occurring in the stage between the initial induction of liver growth and the onset of tumors. The studies on fenofibrate and Wy-14,643 suggest that at least with some compounds a mild toxic process and a prolonged episode of increased cell turnover occur. With other compounds, such as DEHP (62) and α-hexachlorocyclohexane (11), there is no evidence of an effect on cell turnover. Nevertheless, with all these compounds, the liver remains enlarged from the early stages until the appearance of tumors, suggesting that the original demands made on the liver persist. This type of "stress" may involve episodes of proliferative activity

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Table 7 Tumor incidence in rodent bladder by foreign bodies (71)

Substance	Tumor incidence (%)	
	Papilloma	Carcinoma
Paraffin wax[a]	5.4	3.6
Paraffin wax[b]	1.2	1.2
Cholesterol[b]	1.8	9.1
Palmitic acid		14.0
Hexamethylbenzene[b]		11.0
Arachid acid[b]		15.0
Glass beads	1.5	1.5
Calcium oxalate stones[c]		3.0

[a] Pellets after heating to 80°C
[b] Powdered and compressed in tablet machine
[c] Formed during dietary treatment of rats with diethylene glycol

that could easily be missed in commonly undertaken experiments, typically employing infrequent sampling (e.g. 2 or 3 times over 2 years), particularly if the hyperplastic episodes are focal.

BLADDER CHANGES AND TUMORS IN RODENTS

Following the discovery that occupational exposure to β-naphthylamine produced bladder tumors in humans (70), studies were conducted in experimental animals involving the implantation of various test substances into the bladder to gain an insight into the mechanism of bladder tumor induction. However, various workers showed that implantation of foreign bodies into the cavity of the rodent bladder induced transitional-cell or squamous-cell carcinomas even without the addition of a carcinogen (Table 7; 71, 72). Attempts were made to attribute these tumors to the presence of carcinogenic contaminants in implanted materials, but even carefully washed glass beads produced carcinomas (71).

A carcinogenicity study on diethylene glycol is of particular interest, in that when administered in the diet of rats for 2 years, both bladder stones (calcium oxalate) and bladder tumors were observed at a dietary level of 4%, but neither was seen at 2% (72). When the stones were removed, cleaned, and implanted into the urinary bladder of young untreated rats, carcinomas developed in a high proportion of the recipients, clearly showing that the presence of a stone was the determining factor in the production of tumors (72). Following implantation of solid objects into the rat bladder, a strong correlation was found between the occurrence of hyperplasia and metaplasia in the bladder epithelium and the development of bladder carcinomas (73).

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Table 8 Relationship between bladder stones and tumors in mice treated with ESNS (74)

Treatment	Concentration (%)	Bladder pathology		
		Epithelial hyperplasia	Stones	Tumors
ESNS	0.01	18[a]/26	13/26	7/26
ESNS + NH_4Cl	0.01 + 1.0	22[b]/26	–	–

[a] Marked [b] Mild
ESNS = 4-Ethylsulfonylnaphthalene-1-sulfonamide

Further support for a correlation between stone formation, hyperplasia, and carcinoma was obtained in a study on 4-ethylsulfonyl-naphthalene-1-sulfonamide (ESNS) (74). ESNS is a potent bladder carcinogen in mice and tumors were always accompanied by the presence of calculi in the bladder (74). The alkaline urine was thought to be an important contributory factor in stone formation. When the alkalinity of the urine was corrected by the administration of ammonium chloride together with ESNS in the drinking water no bladder stones or bladder cancer developed (Table 8; 74). Moreover, the addition of ammonium chloride also led to the virtual disappearance of the hyperplastic response (Table 8). This suggests that the continued presence of severe hyperplasia is important in tumor development.

Saccharin, which is not mutagenic (75, 76), provides another example of tumor production in the bladder through a nongenotoxic mechanism. Sodium saccharin induces sustained increases in the relative weight and epithelial hyperplasia incidence of the bladder, accompanied by persistent diuresis and these changes have been causally associated with eventual bladder tumor formation in the rat (77).

Terephthalic acid and melamine also induce both tumors and stone formation in the bladder. These compounds are nongenotoxic but induce marked changes in the transitional epithelium including hyperplasia, papilloma and squamous metaplasia, and eventually transitional-cell and squamous-cell tumors (78).

The evidence suggests that urothelial carcinoma develops from a step-wise process that commences with hyperplasia and metaplasia and culminates in the production of a malignant growth (72–74) so that persistent hyperplasia, particularly if graded as severe, must be regarded as a precursor of such tumor development.

FORESTOMACH CHANGES AND TUMORS IN RODENTS

The forestomach of rats and mice is lined with squamous epithelium that histologically, bears a close resemblance to that of skin. Although this an-

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Table 9 Relationship between chain length of fatty acids added to the diet of rats and forestomach proliferative lesions (86)

Fatty acid[a]	Number of C atoms	Hyperplasia	Papillomatous growth	Carcinoma
Propionic	3	+	+	+
Butyric	4	+	+	
Valeric	5	+	+	
Caproic	6	–	–	
Capric	11	–	–	
Lauric	12	–	–	
Palmitic	16	–	–	

+ = Present – = Absent

[a] Propionic acid administered up to 4% in diet for 2 years; all other fatty acids fed up to 10% of diet for 150 days

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atomical region has no counterpart in the human stomach, induction of tumors in this region cannot be dismissed as necessarily being irrelevant to man since the human esophageal epithelium has similarities to the epithelium of the rodent forestomach.

Polycyclic aromatic hydrocarbons, N-methylnitrosoguanidine, and β-propiolactone, all of which are genotoxic, readily produce tumors in the forestomach mucosa of the rat and mouse as well as in the glandular part of the stomach (79, 80). Tumors limited to the forestomach have been induced by *d*-limonene (80) and more recently by butylated hydroxyanisole (BHA) (81) and propionic acid (82). Mutagenicity tests on these three agents were negative (83, 84). Short-term toxicity studies on BHA revealed that at dietary levels capable of inducing marked epithelial hyperplasia stomach tumors developed (84), but at known subcarcinogenic levels no hyperplasia was observed (85). Studies on a series of fatty acids showed that propionic, butyric, and valeric acids all induced hyperplasia and papillomatous growths in the rat forestomach but longer-chain fatty acids produced neither lesion (Table 9; 86). At known subcarcinogenic dose levels of propionic acid no hyperplasia was seen (P. Grasso, unpublished data).

The results of these studies underline the importance of sustained hyperplasia as a determining factor in carcinogenesis by nongenotoxic agents.

ENDOCRINE CHANGES AND TUMORS IN RODENTS

A causal connection between the administration of hormones and cancer induction in experimental animals has been recognized since Lacassagne (87) induced mammary adenocarcinomas in mice by the long-term administration of estrone benzoate. Since then many studies have shown that endogenous and exogenous hormones as well as hormone-mimetic agents (e.g. di-

Table 10 Examples of hormonal imbalance and endocrine tumor development in rodents

Treatment	Excess hormone	Tumor site
Iodine-deficiency, goitrogens	TSH	Thyroid
Estrogens	Prolactin	Mammary gland
Ovarian transplant[a]	Gonadotrophins	Ovaries (granulosa cells)
Histamine H_2-antagonists	Gastrin	Stomach (ECL cells)
Estrogens	Prolactin[b]	Pituitary
Chronic thyroid deficiency	TSH[b]	Pituitary

TSH = Thyroid-stimulating hormone
[a] Implantation of ovary into spleen of ovariectomized rats
[b] Tumors result from excessive production of hormone-secreting cells in the pituitary

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ethylstilbestrol) can cause tumors. The conditions necessary for tumor development are not clearly defined but generally involve a serious imbalance in the normal feedback control mechanism between the hypothalamus, pituitary, and target endocrine organ such that one or more hormones would attain "supraphysiological" levels. The subject is vast and complex and only a few examples (Table 10) are given here to illustrate the fact that pathological changes often precede the production of tumors induced by excessive hormone stimulation.

An important example of hormonal carcinogenesis is the induction of thyroid adenomas and carcinomas in rodents. These tumors can be induced in mice and rats by long-term administration of a low-iodine diet (88), naturally occurring or synthetic goitrogens (e.g. methyl- or propylthiouracil) or thyroid-stimulating hormone (TSH) (89–92).

IARC (93) summarized the likely mechanism of tumor production essentially as follows: "Thyroid tumors have been induced by various anti-thyroid substances, by low iodine diets and subtotal thyroidectomy. In the case of their induction in animals by continuous feeding of a low iodine diet, causation is attributed to hypersecretion of TSH acting upon the hypofunctional thyroid which displays a disturbed synthesis of thyroid hormone due to decreased availability of inorganic iodine. This clearly indicates the existence of an indirect mechanism leading to thyroid carcinogenesis, the primary cause being a hormonal imbalance of the hypothalamo-pituitary-thyroid system. Anti-thyroid substances suppress the rate of synthesis of thyroxine and this similarly leads to a hormonal imbalance and thyroid tumor induction."

Imbalance of thyroid hormones causes marked pathological changes. In mice and rats chronically exposed to a low-iodine diet, changes progress from moderate to extensive hyperplasia to parenchymatous or colloid goiter and eventually to thyroid adenomas and carcinomas. These slow-growing tumors

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are hormone-dependent (requiring TSH for their maintenance) during the early stages but can metastasize to the lung and lymph nodes (94). A similar progression of lesions has been observed in tumor development brought about by the administration of goitrogens and TSH (95, 96).

Another well-studied example of hormonal carcinogenesis is the induction of mammary gland tumors by estrogens. Mammary tumors have been induced by long-term administration of estrogens (synthetic or naturally occurring) in mice and in other rodent species treated with naturally occurring estrogens (17β-estradiol and estrone) (97).

These hormones are instrumental in transforming normal mammary ducts of alveolar epithelium to foci of nodular hyperplasia and then to carcinomas in mice (97). Estrogens alone are critical in the formation of nodules and further progression to carcinomas requires the presence of other hormones, particularly prolactin and growth hormone (98, 99).

Pituitary tumors can also be induced in rats and mice by the administration of estrogens (94, 100). The initial hyperplastic lesion, reflecting an increase in prolactin-secreting cells, progresses to an adenoma (100). Pituitary tumors have been reported in mice rendered hypothyroid either by the administration of large doses of radioiodine, by surgical removal of the thyroid, or by an iodine-deficient diet (94, 100, 101).

Imbalance in the pituitary-gonadal hormone axis in either sex can also lead to tumors, e.g. ovarian granulosa cell tumors from over-production of gonadotrophins. A study in 1949 showed that when both ovaries were surgically removed and one was then implanted into the spleen of the same rat, granulosa cell tumors developed within the implanted ovary. It was not shown whether the expected preceding hyperplasia actually occurred (102).

In male rats, Leydig cell tumors arise from an imbalance of gonadotrophic hormones induced by transplanting the infantile rat testis to the spleen of castrated rats, subtotal castration, or systemic administration of gonadotrophins. Foci of Leydig cell hyperplasia were often observed in these studies, eventually progressing to neoplasms (94, 103).

These studies indicate that imbalance in the pituitary-thyroid, pituitary-mammary or pituitary-gonadal axis can lead to tumor development, via the intermediate stage of hyperplasia.

A more recent example of hormonal carcinogenesis is that of carcinoid tumors of the stomach. These tumors have been induced in rats and mice by histamine H_2-receptor antagonists, such as omeprazole and oxmetidine, and by the hypolipidemic agent, ciprofibrate. These compounds also induce a sustained 3–4 fold increase in plasma gastrin and a suppression of HCl secretion in rodents (104–107). Moreover, at doses not producing marked increases in plasma gastrin or enterochromaffinlike cell (ECL) hyperplasia, no tumors of the glandular stomach arose, whereas at higher doses both of

these early effects and tumors occurred, thus indicating the existence of a threshold dose (104).

Detailed study of the early stages of treatment revealed an increase not only of the gastrin-secreting cells but also of a variety of other enterochromaffin-like cells, which so far have not been clearly characterized. Carcinoid tumors arise from this latter group of cells (108, 109).

Thus, the findings in studies on hormonal carcinogenesis have parallels with the experience gained at other sites. Tumors are produced after a prolonged insult of the target endocrine tissue by agents that are inherently devoid of any mutagenic activity. In most cases the presence of such an insult is manifested by a hyperplastic response from the earliest stages of treatment.

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SKIN CHANGES AND TUMORS IN MICE

Mouse skin has been employed for over 50 years as a model to detect topically active carcinogens. Numerous genotoxic compounds but relatively few nongenotoxic compounds are known to induce mouse skin tumors.

In 1925 (110), squamous cell carcinomas were induced by long-term application of concentrated solutions of nongcnotoxic HCl or NaOH to the shaved skin of mice. The results were treated with caution, possibly because of suspected contamination by chemical carcinogens. More recently, however, Schmahl (111) reproduced these results using KOH or NaOH under strictly controlled conditions. Both studies employed almost daily application of the compound and both studies reported the occurrence of skin ulceration that persisted until tumor onset. Thus, prolonged injury to mouse skin can predispose to tumor production. Although neither study reported the presence of hyperplasia, it is inconceivable that none occurred, particularly at the edges of the lesion where attempts at repair must have been continually taking place.

With the nongenotoxic surface-active agents Tweens and Spans, most did not produce any skin tumors in carcinogenicity tests carried out according to the standards prevalent in the 1950s (112) but some did produce a few papillomas. Croton oil, thought at one time to be a "pure" skin tumor promoter, was shown to induce a low incidence of papillomas and carcinomas when applied frequently for several months (113). Its active principle, 12-0-tetradecanoylphorbol-13-acetate (TPA), which is now commonly employed as a promoting agent instead of croton oil, also produced a low incidence of papillomas (114).

The relationship between skin damage and skin tumor development by nongenotoxic hydrocarbons was further evidenced by a study demonstrating that certain fractions obtained during the fractional distillation of crude oil (namely, middle distillates) produced a high incidence of both benign and malignant tumors following lifetime painting of mouse skin 2–3 times weekly

(115). Some of the fractions, akin in composition to that of white spirit or kerosene, were found to be devoid of carcinogenic polycyclic aromatic hydrocarbons. In a short-term mouse-skin painting study involving thrice-weekly applications, these fractions produced massive skin damage (severe necrosis and regenerative hyperplasia) within one week of treatment (115). Over the 6-week duration of the study, mitotic activity was considerably increased. This extremely high regenerative activity would provide a powerful hyperplastic stimulus to any cells within the epidermis, rendering them more vulnerable to transformation by some environmental or endogenous mutagen or through some other means.

KIDNEY CHANGES AND TUMORS IN RODENTS

Tumor induction in the kidney following chronic injury attracted little attention until the recent discovery that several nongenotoxic agents that cause the accumulation of $\alpha_{2\mu}$-globulin in the renal tubules of the rat kidney also induce adenocarcinomas (116). Hitherto, the only evidence of persistent damage preceding the development of renal cancer induced by nongenotoxic agents came from nephrotoxicity studies of water-soluble lead salts (mainly acetate and subacetate) (117, 118), which are nongenotoxic (119, 120).

Renal tumors have been induced in both sexes of rats and mice given lead compounds in the diet or subcutaneously (121–126). Study of their histogenesis (127, 128) showed that tumors occurred usually in one and occasionally both kidneys and appeared either as small nodules or as lobulated masses. Histologically, the tumors were diagnosed as adenomas or adenocarcinomas. The adenomas were usually small and made up of cells closely resembling those of the tubular epithelium. The adenocarcinomas were, on the other hand, made up of "clear" cells that despite their large size and presence of an "empty" central area in the cell contained several morphological features (particularly at the electron microscopic level) present also in the tubular cell, and Ito and colleagues (127, 128) concluded therefore that the renal tumors induced by soluble lead salts arose from the renal tubular epithelium. Renal damage, consisting of degenerative and proliferative changes in the proximal and distal tubular cells, was observed in the nonneoplastic regions (128), indicating that these proliferative lesions were precursors of the renal tumors.

In studies of the lesions preceding tumor development (117, 118), a single subcutaneous dose of 1 mg or more of lead acetate to rats induced a burst of mitotic activity within 36–48 hours in the proximal tubular cells, the site of origin of lead-induced adenocarcinomas. Weekly intraperitoneal doses of 1–7 mg of lead acetate resulted in a 15-fold increase in the uptake of ^{3}H-thymidine in the proximal and distal tubular cells in rats at 6 months. Although no such assays on cell replication were undertaken before 6 months,

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the above effect of a single dose on mitotic activity coupled with the massive increase in ^{3}H-thymidine uptake suggests the presence of a progressive and sustained increase in renal tubular hyperplasia. Not unexpectedly, tumors were not observed in the 6-month study; however, there were focal proliferative areas that may be the immediate precursors of tumors.

The studies of Ito et al (127, 128) and Choie & Richter (117, 118) leave little doubt that administration of lead salts produces sustained proliferative activity in the renal tubular epithelial cells of the rat and appears to be causally related to the production of neoplasia in this organ.

Lead salts produce nuclear inclusions in the rat (129) that may represent a form of degenerative change since cells containing such inclusions do not appear to proliferate on continued administration (117, 118). No such inclusions have been described in the neoplasms induced by lead salts.

More recently, another type of inclusion body has provoked interest. Unlike lead inclusions, these novel bodies are cytoplasmic and consist of an accumulation of a protein in the lysosomes of renal tubular cells. The protein has a molecular weight of 18,700 (116) and is a $\alpha_{2\mu}$-globulin. It is synthesized in the liver of male rats and in much lesser quantities in the salivary gland of female rats (130). In male rats, its synthesis is under multihormonal control with androgens having a predominating influence (131, 132).

The importance of this protein in renal carcinogenesis was recognized by the discovery that various nongenotoxic agents, including unleaded gasoline, decalin, *d*-limonene, isophorone, and 1,4-dichlorobenzene, produced renal tumors in male (but not female) rats (116, 132–134) and also marked accumulation of $\alpha_{2\mu}$-globulin in the renal tubules. Under normal conditions, approximately 60% of this protein, which passes through the glomerular filter, is reabsorbed by the renal tubules and hydrolyzed by lysosomal enzymes to its constituent amino acids, which are then absorbed into the circulation. The above-mentioned chemicals bind to the protein; the conjugate is less easily digested by lysosomal enzymes, thereby resulting in its accumulation within the lysosome.

Further studies (116, 134a) have shown that the extent of accumulation of $\alpha_{2\mu}$-globulin is dose-related, the greater the dose of the protein-binding chemical the greater the density of these eosinophilic inclusions in the P-2 segment of the kidneys. There was also a dose-related increase in cell turnover rate (up to sixfold) and in the degeneration and necrosis of individual cells lining the P-2 segment of the proximal tubule as well as in the formation of granular casts at the junction of the loop of Henle and the P-3 segment. These pathological changes persist in an "exposure-related extent" for as long as the rat produces $\alpha_{2\mu}$-globulin and the increase in cell proliferation was considered (116, 134a) to act as a "tumor promoter by clonally expanding spontaneously initiated cells in the kidney."

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OTHER SITES—CHANGES AND TUMORS IN RODENTS

Respiratory Tract

Growing evidence indicates that nongenotoxic mechanisms of carcinogenic action may also operate in the respiratory tract and the intestine of rodents, though the evidence to date is much weaker than that obtained at the other sites, discussed previously in this review.

In the case of formaldehyde, a strong tissue irritant that acts on the upper respiratory tract, an inhalation study in rats revealed a 40% incidence of squamous cell carcinoma at the highest atmospheric concentration tested and incidences of 15% and 0% at about half and one-eighth of this concentration, respectively (135). Unfortunately, no correlation was attempted between the severity of the irritant reaction and the development of tumors but it is evident from other studies that squamous metaplasia of the respiratory epithelium of the upper respiratory tract can occur at the two highest concentrations, which were carcinogenic.

It can be argued that since formaldehyde is genotoxic, no firm association can be made between the type and degree of tissue damage and the ultimate development of squamous cell carcinoma, because the occurrence of genetic damage cannot be excluded as a possible cause of development. Nevertheless, with the steep dose-response relationship and the virtual absence of adenocarcinomas or esthesioepitheliomas, the tumor-inducing role played by the compound's genotoxicity is unlikely to be a major one and it is far more likely that the pathological changes produced acted as a precursor of tumor development.

The lung is another site exhibiting tissue injury, possible leading to tumor development. Thus, in rats inhaling a high concentration of titanium dioxide powder, the lung alveoli became full of macrophages that had engulfed these particles (136). Later, squamous cysts and squamous cell carcinomas developed. Similar pathological changes occurred when rats inhaled diesel exhaust fumes containing a thousand times more carbon particles than "curbside" concentrations of diesel exhaust fumes inhaled by humans (137). At such high concentrations, within a few days the lung alveoli became full of macrophages containing carbon particles. Later, squamous cell carcinomas appeared in the bronchi (138). The carbon particles from diesel exhaust fumes contain small concentrations of polycyclic aromatic compound carcinogens and these may well have played a role in tumor development. Nevertheless, at concentrations of diesel exhaust fumes that do not produce overloading of the alveoli with macrophages, no tumors arose, clearly suggesting an important role for the macrophage overloading in the neoplastic process (138, 139).

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Intestine

Yet another site of interest is the intestine of the rat where squamous metaplasia and carcinoma developed following administration of degraded carregeenan at 4% of the diet (140). Squamous metaplasia occurred in the lower part of the colon after a few weeks treatment and carcinomas developed in the same region, presumably from the area of squamous metaplasia (141). No systematic studies have yet been conducted to connect these two lesions; nevertheless, such an association would appear to be plausible on general pathological considerations.

SUSTAINED TISSUE HYPERPLASIA AND CANCER DEVELOPMENT IN HUMANS

The evidence available in rodent studies on various nongenotoxic chemicals supports the view that sustained tissue damage occurring in the early/intermediate stages of exposure are causally related to tumor development at various sites (Table 11). The question has to be considered whether persistent tissue damage in humans similarly acts as a precursor of tumor development, notwithstanding the limited database in humans, compared with the rodent.

It has long been known that the incidence of squamous-cell carcinoma is high in patients with chronic ulcers, such as varicose or tropical ulcers, and that such tumors tend to develop in the epithelium around the ulcer (142, 143). This epithelium exhibits a marked hyperplastic reaction and is termed "pseudo-epitheliomatous hyperplasia". Squamous-cell carcinoma is also quite common at the edges of chronic ulcers that result from burns (Majolin's ulcer), at the edges of tuberculous sinuses, and of lupus vulgaris, a chronic tuberculous lesion of the skin (142, 143). With the advent of powerful antituberculous drugs and an improvement in the treatment of burns and ulcers, these types of cancers are now much less frequently encountered, with the result that sound documentary evidence on the relationship is now virtually impossible to obtain. Nevertheless, the evidence, such as it is, does provide some indication that chronic lesions associated with repeated injury and repair of the skin can lead to cancer in humans.

Conclusive evidence establishes that liver cancer is 10 times more likely in humans with cirrhotic than noncirrhotic livers (31, 144). This association is evident when the cause of liver cirrhosis is known, as in the alcoholic or postnecrotic form of disease, or when it is cryptogenic. Some types of cirrhosis are more prone than others to an exceptionally high tumor incidence: thus, hemochromatosis is reportedly 3 or 4 times more likely than other types of cirrhosis to lead to the development of hepatocellular carcinoma (31).

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Table 11 Evidence for causal association of chronic tissue injury and neoplasia in rodents treated with nongenotoxic carcinogens—some examples

Test agent	Early/intermediate changes	Tumor site	Evidence of causal association			Reference
			Threshold dose	Regression	Inhibition	
Solid implants (subcutaneous)	Fibrous reaction	Connective tissue	Yes[a]	Yes		17
Food colorings (subcutaneous)	Fibrous reaction	Connective tissue	Yes[b]			30
CCl_4 or $CHCl_3$	Necrosis and regenerative hyperplasia	Liver	Yes			33, 37
Ponceau MX	Lysosomal disturbance	Liver	Yes			46
DEHP or fenofibrate	Liver enlargement, peroxisomal proliferation, lipofuscin accumulation	Liver	Yes			59, 60
Diethylene glycol	Hyperplasia/stones	Bladder	Yes			72
ESNS[c]	Hyperplasia/stones	Bladder			Yes[d]	74
BHA or propionic acid	Hyperplasia	Forestomach	Yes			81, 82
H_2-antagonists	Hypergastrinemia/hyperplasia of ECL cells	Glandular stomach	Yes			104

[a] Threshold relates to size
[b] Threshold relates to concentration injected
[c] 4-Ethylsulfonylnaphthalene-1-sulfonamide
[d] Correction of alkaline urine with NH_4Cl

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The introduction of oral contraceptives in the 1960s increased the incidence of focal nodular hyperplasia and hepatocellular adenoma in women users, especially among those less than 35 years old (145, 146). Elevated liver enzyme levels present in the serum of users suggested the presence of liver cell damage (147). Enzyme levels returned to normal levels following drug withdrawal. It is most unlikely that the steroids cause overt liver damage but that subcellular damage, evidenced by the rise in liver enzymes, was effective in producing the neoplastic transformation in women. Reductions in the hormone content and duration of treatment with oral contraceptives over the years have been associated with a reduction in the incidence of liver tumor risk (148).

Further evidence of the role of sustained hyperplasia in tumor development in humans comes from thyroid dyshormonogenesis, a rare congenital disorder. In this condition, the defect in thyroid hormone synthesis may be due to an impairment of either the iodide pump or thyroglobulin synthesis, or to the absence of intrathyroid deiodinase. The resultant rise in TSH levels leads to the development of an enlarged hyperplastic thyroid, which, if left untreated, eventually progresses to nodular goiter and occasionally to carcinoma (149).

Asbestos provides another example of chronic tissue damage that is associated with cancer in humans. This agent is inhaled in the form of small needlelike crystals that can find their way to the alveoli where they form the so-called asbestos bodies. Two principal types of tissue reaction have been identified: a lung fibrosis with considerable involvement of the bronchioles, which become dilated and lined by tall or flattened epithelial cells or by goblet cells, and a gross thickening of the pleura with formation of plaques. Two types of lung tumors are associated with exposure to asbestos: squamous-cell carcinoma and mesothelioma. The squamous-cell carcinomas are thought to be caused by the impaction of the larger asbestos fibers in the smaller bronchi and bronchioles with consequent damage to the lining epithelium. In contrast, the mesotheliomas appear to be more closely associated with the presence of smaller asbestos fibers leading to the formation of pleural plaques (150). Although these correlations may be fortuitous, the possibility that the tissue damage induced by asbestos is the determining factor in tumor development cannot be excluded, particularly since there is no convincing evidence to indicate that asbestos is mutagenic (151, 152).

The lung tumors observed in asbestosis may well have a mixed etiology because they are more prevalent in smokers with asbestosis than in nonsmokers with the disease. Nevertheless, smoking may have considerably exacerbated the fibrotic lesions, thus enhancing the possibility of tumor development.

In all of the above cases, tumors have developed in tissues following a prolonged period during which the cells suffer injury and there is a continuous

stimulus for the cells to replicate---exactly the conditions seen with rodents that develop tumors via nongenotoxic mechanisms.

RELEVANCE TO HUMANS OF RODENT TUMORS INDUCED BY NONGENOTOXIC CARCINOGENS

In the previous section, it was shown that chemically induced cancer in humans can apparently be caused by pathological processes in which no genotoxic agent is involved. It is therefore relevant to assess the significance to humans of the chemically induced tumors by the nongenotoxic mechanisms discussed earlier.

One type of rodent tumor causing past concern in respect to potential human risk is the sarcoma induced by foreign bodies or injection. As mentioned earlier, rodents are particularly prone to the development of this tumor and seemingly a well-characterized tissue reaction predisposes to its development.

Several cases have been reported of malignant tumors appearing after the insertion of silicone or metal implants for orthopedic purposes (153). These include malignant lymphoma following insertion of silicone prostheses, and a hemangioendothelioma, sarcoma, and malignant lymphoma following the internal fixation of tibial fractures. Silastic breast prostheses have been linked with subsequent breast tumors (153).

Diggle (153) reviewed the link between implanted solids and tumor production and concluded that "induction of foreign body sarcomas by surgical implants is in the rare category". There is also the possibility that the sarcomas arising around solid implants in humans may be tumors of spontaneous origin that fortuitously develop close to the implant, so that the production of tumors by solid implants in animals has virtually no relevance for humans (154).

The same conclusion applies to sarcomas that arise at the site of repeated injection of drugs such as insulin (155). Although there are occasional reports of tumors at the site of repeated injection, the frequency of diabetes and insulin treatment is such as to exclude an excess risk of tumors, and a causal association between treatment and injection-site tumors is unlikely. Repeated injections of iron-dextran were thought at one time to be capable of producing sarcoma in humans based on rodent findings, but a causal association was not established in humans and moreover the early granulomatous reaction seen in the rat even at human therapeutic dose levels did not occur in humans (156).

The risk of liver tumor development in women on oral contraceptive steroids and the development of liver tumors in experimental animals, particularly in mice, drew attention to the possibility that nongenotoxic carcinogens may present a carcinogenic risk in this organ to humans. At present, there

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does not appear to be any evidence to indicate that nongenotoxic carcinogens, other than oral contraceptives and androgenic anabolic steroids, present a hepatocarcinogenic risk to humans (69, 157).

Epidemiological studies on phenobarbitone (158) and clofibrate (159), both hepatocarcinogenic in the rodent, have shown no evidence of increased hepatic tumor risk in patients on these drugs. Note that the exposure levels of these drugs in humans is considerably lower than the levels known to produce liver tumors in animals. Admittedly, with the contraceptive steroids, the human dose levels are close to those levels inducing liver tumors in rodents (69). It is clear therefore that for nongenotoxic hepatocarcinogens an adequate margin of safety is essential to be confident that they present a negligible hepatocarcinogenic risk to humans. The safety margin can be enhanced by ensuring that the human exposure level is well below the threshold dose in rodents for sustained tissue damage.

Skin tumors can be readily induced in rodents by nongenotoxic agents, if repeatedly applied at doses high enough to induce severe and persistent tissue damage. Although the mechanism of tumor induction by nongenotoxic chemicals in mouse skin is poorly understood, some evidence suggests that if human exposure to such agents is insufficient to cause skin irritation, then there is little risk of cancer.

The development of mammary tumors in the rodent is associated with the sustained action of elevated prolactin on the mammary gland. Prolactin, which can be increased by estrogens, is not deemed to be a risk factor for mammary cancer in humans as it is in rodents. Epidemiological studies in women on oral contraceptives have failed to show clear evidence of an increased mammary tumor risk (160–162).

The role of TSH in the production of thyroid tumors in humans is still obscured by the failure of epidemiological studies to demonstrate a consistent link between thyroid cancer and endemic goiter, usually associated with iodine deficiency. Nevertheless, sustained hypersecretion of TSH has been suggested as playing a contributory role in the induction of adenoma and carcinoma in endemic goiter. In rodents, the role of TSH in thyroid tumor production is more clearly defined. This species difference in response should strike a cautionary note before assessing the likely human risk of developing thyroid cancer, particularly because some evidence exists that other factors, chiefly genetic, may play an important role in humans (163).

The induction of carcinoid tumors in rats and mice by omeprazole and other H_2-antagonists depends on the production of high levels of gastrin (104–107), as discussed earlier. High levels of gastrin appear to have a similar effect in humans, since carcinoid tumors of the stomach are much more common in patients with pernicious anemia than in the general population (164). In pernicious anemia, blood levels of gastrin are high because of a failure in the

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feedback mechanism due to atrophy of the oxyntic cells of the stomach. Conversely, hypergastrinemia may not be seen in humans at or somewhat above therapeutic dose levels of drugs that are known to cause this effect and eventual stomach carcinoid tumors in rodents. In this situation the risk of such tumors arising under clinical conditions of use is considered negligible.

The presence of spontaneous bladder stones in humans does not appear to be a risk factor for bladder tumor development (9). Hence, the induction of bladder tumors in rodents by the mechanism involving stone formation notably at high doses does not appear to have any relevance for humans.

The induction of renal tumors by various compounds, seemingly involving the $\alpha_{2\mu}$-globulin nephropathy mechanism was discussed earlier. This appears to be sex- and species-specific in that only the male rat and not the female rat nor both sexes of mice developed renal tumors. As humans do not produce $\alpha_{2\mu}$-globulin it is unlikely that such compounds would present a nephrocarcinogenic risk to them (116).

SUMMARY AND CONCLUSIONS

This review has focused on the importance of cellular proliferation in neoplasia induced by nongenotoxic carcinogens and has not attempted to address the mechanisms involved in the progression from the hyperplastic to the neoplastic state. Major advances in this area are likely in the next decade and should explain how cells in an abnormally high proliferative state are rendered more vulnerable either to the action of endogenous or environmental mutagens or to defects in cell reproduction, thus providing the stimulus for progression to neoplasia (165).

In this review evidence has been presented to support the hypothesis that sustained tissue damage induced by nongenotoxic compounds in rodents predisposes to tumor development. The evidence has been obtained by drawing from the published findings of experimental rodent studies conducted over the past 50 years.

The experience gained at various sites in the rodent including the connective tissue, liver, bladder, and forestomach shows the existence of a threshold dose for various test materials/agents, below which neither sustained tissue damage nor tumor induction occurs but above which level both effects are manifest (Table 11). Taken collectively, an overall picture emerges that sustained cell proliferation renders various sites in the rodent vulnerable to tumor development.

The validity of this hypothesis is of importance to the evaluation of carcinogenic risk of chemicals to humans. For those nongenotoxic carcinogens known to cause characteristically defined and sustainable tissue damage as a precursor of tumor development in rodents, it should be possible to

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establish a threshold dose below which both effects disappear and upon which a safety margin in humans can be based.

Some limited evidence supports an association between chronic tissue injury and neoplastic development in humans. Note, however, that certain types of induced tissue damage may be rodent-specific and therefore have no relevance for humans.

We conclude that the appearance of persistent tissue damage predisposes to tumor development in rodents exposed to nongenotoxic carcinogens and that systematic studies in rodents should provide a rational basis for arriving at a safety margin in humans exposed to such agents.

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Literature Cited

1. Yamagiwa, K., Ichikawa, K. 1918. Experimental study of the pathogenesis of carcinoma. *J. Cancer Res.* 3:1–29
2. Kennaway, E. L. 1925. Experiments on cancer producing substances. *Br. Med. J.* 69:2–4
3. Leitch, A. 1922. Paraffin cancer and its experimental production. *Br. Med. J.* 2:1104–6
4. Woglom, W. H. 1926. Experimental tar cancer. *Arch. Pathol.* 2:534–76
5. Cook, J. W., Hewett, C. L., Hieger, I. 1933. The isolation of cancer producing hydrocarbons from coal tar. Parts I, II, III. *J. Chem. Soc.*, 395
6. Berenblum, I., Schoental, R. 1946. Carcinogenic constituents of coal-tar. *Br. J. Cancer* 1:157–65
7. Douglas, G. R. L., Blakey, D. H., Clayson, D. B. 1988. Genotoxicity tests as predictors of carcinogenesis: An analysis. *Mutat. Res.* 196:83–93
8. Perera, F. P. 1984. The genotoxic/epigenetic distinction: Relevance to cancer policy. *Environ. Res.* 34:175–91
9. Clayson, D. B. 1989. Can a mechanistic rationale be provided for non-genotoxic carcinogens identified in rodent bioassays? *Mutat. Res.* 221:53–67
10. Kato, R., Takanaka, A., Onoda, K. I. 1970. Studies on age difference in mice for the activity of drug metabolizing enzymes of liver microsomes. *Jpn. J. Pharmacol.* 20:572–76
11. Schulte-Hermann, R. 1974. Induction of liver growth by xenobiotic compounds and other stimuli. *CRC Crit. Rev. Toxicol.* 3:97–158
12. Turner, F. C. 1941. Sarcomas at sites of subcutaneously implanted bakelite disks in rats. *J. Natl. Cancer Inst.* 2:81–83
13. Oppenheimer, B. S., Oppenheimer, E. T., Stout, A. P. 1948. Sarcoma induced in rats by implanting cellophane. *Proc. Soc. Exp. Biol. Med.* 67:33–34
14. Oppenheimer, B. S., Oppenheimer, E. T., Danishefsky, I., Stout, A. P., Eirich, F. R. 1955. Further studies of polymers as carcinogenic agents in animals. *Cancer Res.* 15:333–40
15. Oppenheimer, B. S., Oppenheimer, E. T., Stout, A. P., Danishefsky, I., Willhite, M. 1959. Studies of the mechanism of carcinogenesis by plastic films. *Acta Unio Int. Contra Cancrum* 15:659–63
16. Oppenheimer, E. T., Willhite, M., Stout, A. P., Danishefsky, I., Fishman, M. M. 1964. A comparative study of the effects of embedding cellophane and polystyrene films in rats. *Cancer Res.* 24:379–82
17. Bischoff, F., Bryson, G. 1964. Carcinogenesis through solid state surfaces. *Prog. Exp. Tumor Res.* 5:85–133
18. Brand, K. G., Buoen, L. C., Johnson, K. H., Brand, I. 1975. Etiological factors, stages, and the role of the foreign body in foreign body tumorigenesis: A Review. *Cancer Res.* 35:279–86
19. Nothdurft, H. 1956. Experimentelle Sarkomauslösung durch eingeheilte Fremdkörper. *Strahlentherapie* 100:192–210
20. Nothdurft, H. 1961. Sarkomerzeugung bei Ratten durch implantierte Fremdkörper. *Ther. Monatsh.* 8:262
21. Oppenheimer, B. S., Oppenheimer, E. T., Stout, A. P., Willhite, M., Danishefsky, I. 1958. The latent period in carcinogenesis by plastics in rats and its relation to the presarcomatous stage. *Cancer NY* 11:204–13
22. Golberg, L. 1963. Die Wirkung von Eiseninjektionen im Tierversuch. *Arzneim. Forsch.* 13:939–45
23. Baker, S. B. de C., Golberg, L., Mar-

Annu. Rev. Pharmacol. Toxicol. 1991.31:253-287. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

tin, L. E., Smith, J. P. 1961. Tissue changes following injection of iron-dextran complex. *J. Pathol. Bacteriol.* 82:453–70
24. Nishiyama, Y. 1938. Experimentelle Erzeugung des Sarkomas bei Ratten durch wiederholte Injektionen von Glucoselösung. *Gann* 32:85–99
25. Nonaka, T. 1938. The occurrence of subcutaneous sarcomas in the rat after repeated injections of glucose solution. *Gann* 32:234–35
26. Tokoro, Y. 1940. Über die artificielle Erzeugung des Sarkoms bei den weissen Ratten mittels konzentrierter Kochsalzlosung. *Gann* 34:149–55
27. Boyland, E. 1958. The biological examination of carcinogenic substances. *Br. Med. Bull.* 14:93–98
28. Gangolli, S. D., Grasso, P., Golberg, L. 1967. Physical factors determining the early local tissue reactions produced by food colourings and other compounds injected subcutaneously. *Food Cosmet. Toxicol.* 5:601–21
29. Grasso, P., Golberg, L. 1966. Subcutaneous sarcoma as an index of carcinogenic potency. *Food Cosmet. Toxicol.* 4:297–320
30. Grasso, P., Gangolli, S. D., Golberg, L., Hooson, J. 1971. Physicochemical and other factors determining local sarcoma production by food additives. *Food Cosmet. Toxicol.* 9:463–78
31. MacSween, R. N. M., Scott, A. R. 1973. Hepatic cirrhosis: A clinico-pathologic review of 520 cases. *J. Clin. Pathol.* 26:936–42
32. Edwards, J. E., Dalton, A. J. 1942. Induction of cirrhosis of the liver and of hepatomas in mice with carbon tetrachloride. *J. Natl. Cancer Inst.* 3:19–41
33. Edwards, J. E., Heston, W. E., Dalton, A. J. 1942. Induction of the carbon tetrachloride hepatoma in strain L mice. *J. Natl. Cancer Inst.* 3:297–301
34. Reuber, M. D., Glover, E. L. 1968. Carbon tetrachloride induced cirrhosis: Effects of age and sex. *Arch. Pathol.* 85:275–80
35. Simmon, V. F., Kauhanen, K., Tardiff, R. G. 1977. Mutagenic activity of chemicals identified in drinking water. In *Progress in Genetic Toxicology: Development in Toxicology and Environment Science,* ed. D. Scott, B. A. Bridges, F. H. Sobels, 2:249–58. New York: Elsevier North-Holland Biomed.
36. Rickart, R., Appel, K. E., Schwarz, M., Stockle, G., Kunz, W. 1980. Correlation between primary effects of xenobiotics on liver cells in vitro and their mutagenicity and carcinogenicity in vivo. In *Short Term Mutagenicity Test System for Detecting Carcinogens,* ed. K. H. Norpoth, R. C., Gaer, p. 179. Berlin: Springer-Verlag
37. Eschenbrenner, A. B., Miller, E. 1945. Induction of hepatomas in mice by repeated oral administration of chloroform, with observations on sex differences. *J. Natl. Cancer Inst.* 5:251–55
38. Reitz, R. H., Quast, J. F., Stott, W. T., Watanabe, P. G., Gehring, P. J. 1980. Pharmacokinetics and macromolecular effects of chloroform in rats and mice: implications for carcinogenic risk estimation. In *Water Chlorination, Environmental Impact and Health Effects,* ed. R. L. Jolley, W. A. Brungs, R. B. Cumming, 3:983–93. Ann Arbor, MI: Sci. Publ.
39. Van Abbe, N. J., Gren, T. J., Jones, E., Richold, M., Roe, F. J. C. 1982. Bacterial mutagenicity studies on chloroform *in vitro*. *Food Chem. Toxicol.* 20:557–62
40. Grasso, P., Hinton, R. 1990. Evidence for and possible mechanisms of nongenotoxic carcinogenesis in rodent liver. *Mutat. Res.* In press
41. de Duve, C., Wattiaux, R. 1966. Functions of lysosomes. *Annu. Rev. Physiol.* 28:435–92
42. Kerr, J. F. R. 1967. Lysosome changes in acute liver injury due to heliotrine. *J. Pathol. Bacteriol.* 93:167–74
43. Arstila, A. U., Trump, B. F. 1968. Studies on cellular autophagocytosis. The formation of autophagic vacuoles in the liver after glucagon administration. *Am. J. Pathol.* 53:687–733
44. Crampton, R. F., Gray, T. J. B., Grasso, P., Parke, D. V. P. 1977. Long-term studies on chemically induced liver enlargement in the rat. II. Transient induction of microsomal enzymes leading to liver damage and nodular hyperplasia produced by safrole and Ponceau MX. *Toxicology* 7:307–26
45. Grasso, P., Gray, T. J. B. 1977. Long term studies on chemically induced liver enlargement in the rat. III. Structure and behaviour of the hepatic nodular lesions induced by Ponceau MX. *Toxicology* 7:327–42
46. Grasso, P. 1979. Liver growth and tumorigenesis in rats. *Arch. Toxicol. Suppl.* 2:171–80
47. Lijinsky, W., Reuber, M., Blackwell, B. N. 1980. Liver tumors induced in rats by oral administration of the antihistaminic methapyrilene hydrochloride. *Science* 209:817–19
48. Reznic-Schuller, H. M., Lijinsky, W.

Annu. Rev. Pharmacol. Toxicol. 1991.31:253-287. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

1983. Morphology of early changes in liver carcinogenesis induced by methapyrilene. *Arch. Toxicol.* 52:165–66

49. Cohen, A. J., Grasso, P. 1981. Review of the hepatic response to hypolipidemic drugs in rodents and assessment of its toxicological significance to man. *Food Cosmet. Toxicol.* 19:585–605
50. Staubli, W., Hess, R., Weibel, E. R. 1969. Correlated morphometric and biochemical studies on the liver cell. II. Effects of phenobarbital on rat hepatocytes. *J. Cell Biol.* 42:92–112
51. Feuer, G., Gaunt, I. F., Golberg, L., Fairweather, F. A. 1965. Liver response tests. VI. Application to a comparative study of food antioxidants and hepatotoxic agents. *Food Cosmet. Toxicol.* 3:457–69
52. Gaunt, I. F., Feuer, G., Fairweather, F. A., Gilbert, D. 1965. Liver response tests. IV. Application to short-term feeding studies with butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). *Food Cosmet. Toxicol.* 3:433–43
53. Gilbert, D., Golberg, L. 1965. Liver response tests. III. Liver enlargement and stimulation of microsomal processing enzyme activity. *Food Cosmet. Toxicol.* 3:417–32
54. Gilbert, D., Martin, A. D., Gangolli, S. D., Abraham, R., Golberg, L. 1969. The effects of substituted phenols on liver weights and liver enzymes in the rat: Structure-activity relationships. *Food Cosmet. Toxicol.* 7:603–19
55. Fitzhugh, O. G., Nelson, A. A., Frawley, J. P. 1950. The chronic toxicities of technical benzene hexachloride and its alpha, beta and gamma isomers. *J. Pharmacol. Exp. Ther.* 100:59–66
56. de Duve, C., Baudhuin, P. 1966. Peroxisomes (microbodies and related particles). *Physiol. Rev.* 46:323–57
57. Reddy, J. K., Lalwani, N. D. 1983. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit. Rev. Toxicol.* 12:1–58
58. Bakke, O., Berge, R. K. 1984. Induction of peroxisomal enzymes and palmitoyl CoA hydrolase in rats treated with cholestyramine and nicotinic acid. *Biochem. Pharmacol.* 33:3077–80
59. Price, S. C., Hinton, R. H., Mitchell, F. E., Hall, D. E., Grasso, P., et al. 1986. Time and dose study on the response of rats to the hypolipidemic drug fenofibrate. *Toxicology* 41:169–91
60. Mitchell, F. E., Price, S. C., Hinton, R. H., Grasso, P., Bridges, J. W. B. 1985. Time and dose-response study of the effects on rats of the plasticizer di(2-ethylhexyl)phthalate. *Toxicol. Appl. Pharmacol.* 81:371–92
61. Hartroft, W. S., Porta, E. A. 1972. Observation and interpretation of lipid pigments (lipofuscins) in the pathology of laboratory animals. *CRC Crit. Rev. Toxicol.* 1:397–411
62. Marsman, D. S., Cattley, R. C., Conway, J. G., Popp, J. A. 1988. Relationship of hepatic peroxisome proliferation and replicative DNA synthesis to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and [4-chloro-6 (2,3-xylidino)2-pyrimidinethio]acetic acid (Wy-14,643). *Cancer Res.* 48:6739–44
63. Bentley, P., Bieri, F., Mitchell, F., Walchter, F., Staubli, W. 1987. Investigation on the mechanism of liver tumor induction by peroxisome proliferators. In *Mouse Liver Tumors. Arch. Toxicol. Suppl.* 10:57–161
64. Cattley, R. C., Richardson, K. K., Smith-Oliver, T. T., Popp, J. A., Butterworth, B. F. 1986. Effect of peroxisome proliferator carcinogens on unscheduled DNA synthesis in rat hepatocytes determined by autoradiography. *Cancer Lett.* 33:269–77
65. Conway, J. G., Tomaszewski, K. E., Olson, M. J., Cattley, R. C., Marsman, D. S., et al. 1989. Relationship of oxidative damage to the hepatocarcinogenicity of the peroxisome proliferator di-(2-ethylhexyl)phthalate and Wy-14,643. *Carcinogenesis* 10:513–19
66. Schimke, R. T. 1962. Adaptive characteristics of urea cycle enzymes in the rat. *J. Biol. Chem.* 237:459–68
67. Argyris, T. S. 1971. Additive effects of phenobarbital and high protein on liver growth in immature male rats. *Dev. Biol.* 25:293
68. Tannenbaum, A., Silverstone, H. 1949. The genesis and growth of tumors. IV. Effects of varying the proportion of protein (casein) in the diet. *Cancer Res.* 9:162–73
69. IARC. 1979. Sex hormones (II). In *IARC Monogr. on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, 21:233–78. Lyon, France: IARC

69a. Grasso, P. 1987. Persistent organ damage and cancer production in rats and mice. Mechanisms and models in toxicology. *Arch. Toxicol. Suppl.* 11:75–83

70. Case, R. A. M., Hosker, M. E. 1954. Tumor of the urinary bladder as an occupational disease in the rubber industry in England and Wales. *Br. J. Prev. Soc. Med.* 8:39–50

Annu. Rev. Pharmacol. Toxicol. 1991.31:253-287. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

71. Ball, J. K., Field, W. E. H., Roe, F. J. C., Walters, M. 1964. The carcinogenic and co-carcinogenic effects of paraffin wax pellets and glass beads in the mouse bladder. *Br. J. Urol.* 36:225–38
72. Weil, C. S., Carpenter, C. P., Smyth, H. F. Jr. 1965. Urinary bladder response to diethylene glycol. Calculi and tumors following repeated feeding and implants. *Arch. Environ. Health* 11:569–81
73. Roe, F. J. C. 1964. An illustrated classification of the proliferative and neoplastic changes in mouse bladder epithelium in response to prolonged irritation. *Br. J. Urol.* 36:238–53
74. Flaks, A., Hamilton, J. M., Clayson, D. B. 1973. Effect of ammonium chloride on incidence of bladder tumors induced by 4-ethylsulfonyl naphthelene-1-sulfonamide. *J. Natl. Cancer Inst.* 51: 2007–8
75. IARC. 1980. Some non-nutritive sweetening agents. In *IARC Monogr. on the Evaluation of the Carcinogenic Risk of Chemicals to Humans,* 22:111–70. Lyon, France: IARC
76. IARC. 1987. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.* Suppl. 6: Genetic and Related Effects—An Updating of Selected IARC Monogr. from Vols. 1–42, pp. 488–93. Lyon, France: IARC
77. Anderson, R. L. 1990. Early indicators of bladder carcinogenesis produced by non-genotoxic agents. *Mutat. Res.* In press
78. Heck, D'A., Tyl, R. W. 1985. The induction of bladder stones by terephthalic acid, dimethyl terephthalate and melamine (2,4,6-triamino-S-triazine) and its relevance to risk assessment. *Regul. Toxicol. Pharmacol.* 5:294–313
79. Nagayo, T. 1973. Tumors of the stomach. In *Pathology of Tumors in Laboratory Animals.* Part I: *Tumors of the Rat,* ed. V. S. Turusov, 1:101–18. IARC Sci. Ser. No. 5. Lyon, France: IARC
80. Roe, F. J. C., Field, E. H. 1965. Chronic toxicity of essential oils and certain other products of natural origin. *Food Cosmet. Toxicol.* 3:311–24
81. Ito, N. L., Fukushima, S. L., Hagiwara, A. L., Shibata, M., Ogioso, T. 1983. Carcinogenicity of butylated hydroxyanisole in F344 rats. *J. Natl. Cancer Inst.* 70:343–52
82. von Griem, W. 1985. Tumorigene Wirkung von Propionsauren der Vormagenschleimhaut von Ratten im Futterungsversuch. *Bundesgesundheitsblatt* 28:322–27
83. Basler, A., von der Huede, W., Scheutwinkel, M. 1987. Screening of the food additive propionic acid for genotoxic properties. *Food Cosmet. Toxicol.* 25: 287–90
84. Nera, E. A., Lok, E., Iverson, F. L., Ormsby, E. L., Karpinski, K. F. 1984. Short-term pathological and proliferative effects of butylated hydroxyanisole and other phenolic antioxidants in the forestomach of Fischer 344 rats. *Toxicology* 32:197–213
85. Altmann, H.-J., Wester, P. W., Matthiaschk, G., Grunow, W., Van der Heijden, C. A. 1985. Induction of early lesions in the forestomach of rats by 3-*tert*-butyl-4-hydroxyanisole (BHA). *Food Chem. Toxicol.* 23:723–31
86. Mori, K. 1953. Production of gastric lesions in the rat by diet containing fatty acids. *Gann* 44:421–26
87. Lacassagne, A. 1936. A comparative study of the carcinogenic action of certain estrogenic hormones. *Am. J. Cancer* 28:735–40
88. Axelrod, A. A., Leblond, C. P. 1955. Induction of thyroid tumors in rats by a low iodine diet. *Cancer* 8:339–67
89. Morris, H. P., Dalton, A. J., Green, C. D. 1951. Malignant thyroid tumors in the mouse after prolonged hormonal imbalance during the ingestion of thiouracil. *J. Clin. Endocrinol. Metab.* 11: 1281–95
90. Van Dyke, J. H. 1953. Experimental thyroid tumorigenesis in rats. *Arch. Pathol.* 56:613–28
91. Steinhoff, D., Weber, H., Mohr, U., Boehme, K. 1983. Evaluation of amitrole (aminotriazole) for potential carcinogenicity in orally dosed rats, mice, and golden hamsters. *Toxic. Appl. Pharmacol.* 69:161–69
92. Sellers, E. A., Hill, J. M., Lee, R. B. 1953. Effects of iodine and thyroid on the production of tumors of the thyroid and pituitary by propyl thiouracil. *Endocrinology* 52:188–202
93. IARC. 1974. Some anti-thyroid and related substances, nitrofurans and industrial chemicals. In *IARC Monogr. on the Evaluation of the Carcinogenic Risk of Chemicals to Man,* 7:23–26. Lyon, France: IARC
94. Lupulescu, A. 1983. In *Hormones and Carcinogenesis,* p. 99. New York: Praeger
95. Lacassagne, A. 1948. Hormones and their relationship to cancer. *Schweiz. Med. Wochenschr.* 78:705–8
96. Williams, E. D. 1980. Pathologic and natural history. In *Thyroid Cancer,* ed.

Annu. Rev. Pharmacol. Toxicol. 1991.31:253-287. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

W. Duncan, 73:47–55. Berlin: Springer-Verlag
97. Foulds, L. 1975. *Neoplastic Development*, Vol. 2. New York: Academic
98. Furth, J. 1973. The role of prolactin in mammary carcinogenesis. In *Human Prolactin. Proc. Int. Symp. Human Prolactin, New York*, p. 233. New York: Elsevier
99. Meites, J. 1972. Relation of prolactin and estrogens to mammary tumorigenesis in the rat. *J. Natl. Cancer Inst.* 48:1217–24
100. Furth, J., Nakane, P., Pasteels, J. L. 1976. Tumors of the pituitary gland. In *Pathology of Tumors in Laboratory Animals*. Part 2: Tumors of the Rat, ed. V. S. Turusov, 1:201–38. IARC Sci. Publ. Ser. No. 6. Lyon, France: IARC
101. Bielschowsky, F. 1953. Chronic iodine deficiency as cause of neoplasia in thyroid and pituitary of aged rats. *Br. J. Cancer* 7:203–13
102. Biskind, G. R., Biskind, M. S. 1949. Experimental ovarian tumors in rats. *Am. J. Clin. Pathol.* 19:501–21
103. Mostofi, F. K., Bresler, V. M. 1976. Tumors of the testis. See Ref. 100, pp. 135–60
104. Hirth, R. S., Evans, L. D., Buroker, R. A., Oleson, F. B. 1988. Gastric enterochromaffin-like cell hyperplasia and neoplasia in the rat: An indirect effect of the histamine H_2-receptor antagonist, BL-6341, *Toxicol. Pathol.* 16:273–87
105. Betton, G. R., Dormer, C. S., Wells, T., Pert, P., Price, C. A., et al. 1988. Gastric ECL-cell hyperplasia and carcinoids in rodents following chronic administration of H_2-antagonists SK&F 93479 and oxmetidine and omeprazole. *Toxicol. Pathol.* 16:288–98
106. Spencer, A. J., Barbolt, T. A., Henry, D. C., Eason, C. T., Sauerschell, R. J., et al. 1989. Gastric morphological changes including carcinoid tumors in animals treated with a potent hypolipidemic agent, ciprofibrate. *Toxicol. Pathol.* 17:7–15
107. Larsson, H., Carlsson, E., Mattesson, H., Lundell, L., Sundler, F., et al. 1986. Plasma gastrin and gastric enterochromaffin-like cells activation and proliferation. *Gastroenterology* 90:391–99
108. Poynter, D., Pick, C. R., Harcourt, R. A., Selway, S. A. M., Ainge, G., et al. 1986. Association of long lasting unsurmountable histamine H_2 blockade and gastric carcinoid tumors in the rat. *Gut* 26:1284–95
109. Poynter, D., Selway, S. A. M., Papworth, S. A., Riches, S. R. 1986. Changes in the gastric mucosa of the mouse associated with long lasting unsurmountable histamine H_2 blockade. *Gut* 27:1338–46
110. Narat, J. K. 1925. Experimental production of malignant growths by simple chemicals. *J. Cancer Res.* 9:135–38
111. Schmahl, D. 1984. Carcinogenic activity of KOH and NaOH by topical application to mice. In *Maligne Tumoren—Entstehung, Wachtsum Chemotherapie*, ed. D. Schmahl. *Arzneimittel-Forsch. Suppl.* 21:290–91
112. Setälä, K., Setälä, H., Merenmies, L., Holsti, P. 1957. Untersuchungen über die Tumorauslösende ("tumor promoting") Wirkung einiger Nichtionisierbaren Oberflächenaktiven Substanzen bei Maus und Kaninchen. *Z. Krebsforsch.* 61:534–47
113. Roe, F. J. C., Clack, J. 1963. Two-stage carcinogenesis: Effect of length of promoting treatment on the yield of benign and malignant tumors. *Br. J. Cancer* 17:596–604
114. Nesnow, S., Triplett, L. L., Slaga, T. J. 1982. Comparative tumor initiating activity of complex mixtures from environmental particulate emissions on SENCAR mouse skin. *J. Natl. Cancer Inst.* 68:829–34
115. Grasso, P., Sharratt, M., Ingram, A. J. 1988. Early changes produced in mouse skin by the application of three middle distillates. *Cancer Lett.* 42:147–55
116. Swenberg, J. A., Short, B., Borghoff, S., Strasser, J., Charbonneau, M. 1989. The comparative pathobiology of $\alpha_{2\mu}$-globulin nephropathy. *Toxicol. Appl. Pharmacol.* 97:35–46
117. Choie, D. D., Richter, G. W. 1972. Cell proliferation in rat kidneys after prolonged treatment with lead. *Am. J. Pathol.* 68:359–70
118. Choie, D. D., Richter, G. W. 1972. Cell proliferation in rat kidney by lead acetate and effects of uninephrectomy on the proliferation. *Am. J. Pathol.* 66:265–75
119. IARC. 1980. Some metals and metallic compounds. In *IARC Monogr. on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, 23:325–415. Lyon, France: IARC
120. IARC. 1987. *IARC Monogr. on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Suppl. 7: Overall Evaluations of Carcinogenicity—An Updating of IARC Monogr. Vols. 1–24, pp. 230–32. Lyon, France: IARC
121. Van Esch, G. J., Van Genderen, H., Vink, H. H. 1962. The induction of renal tumors by feeding basic lead acetate to rats. *Br. J. Cancer* 16:289–97

Annu. Rev. Pharmacol. Toxicol. 1991.31:253-287. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

122. Boyland, E., Dukes, C. E., Grover, P. L., Mitchley, B. C. U. 1962. The induction of renal tumors by feeding lead acetate to rats. *Br. J. Cancer* 16:283–88
123. Zawirska, B., Medras, K. 1968. Tumors and disorders of porphyrin metabolism in rats with chronic lead intoxication. *Zentralbl. Allg. Pathol. Pathol. Anat.* 111:1–112
124. Van Esch, G. J., Kroes, R. 1969. The induction of renal tumors by feeding basic lead acetate to mice and hamsters. *Br. J. Cancer* 23:765–71
125. Roe, F. J. C., Boyland, E., Dukes, C. E., Mitchley, B. C. V. 1965. Failure of testosterone or xanthopterin to influence the induction of renal neoplasms by lead in rats. *Br. J. Cancer* 19:860–66
126. Furst, A., Schlauder, M., Sasmore, D. P. 1976. Tumorigenic activity of lead chromate. *Cancer Res.* 36:1779–83
127. Ito, N., Haisa, Y., Kamamoto, Y., Makiura, S., Sugihara, S., et al. 1971. Histopathological analysis of kidney tumors in rats induced by chemical carcinogens. *Gann* 62:435–44
128. Ito, N. 1973. Experimental studies on tumors of the urinary system of rats induced by chemical carcinogens. *Acta Pathol. Jpn.* 23:87–109
129. Shakerin, M., Paloucek, J. 1965. Intranuclear inclusions and renal tumors in rats fed lead subacetate. *Lab. Invest.* 14:592
130. Neuhaus, O. W., Flory, W., Biswas, N., Hollerman, C. E. 1981. Urinary excretion of $\alpha_{2\mu}$-globulin and albumin by adult male rats following treatment with nephrotoxic agents. *Nephron* 28:133–40
131. Vandoren, G., Mertens, B., Heyns, C., Van Baelen, H., Rombauts, W., et al. 1983. Different forms of $\alpha_{2\mu}$-globulin in male and female rat urine. *Eur. J. Biochem.* 134:175–81
132. Kitchen, D. N. 1984. Neoplastic renal effects of unleaded gasoline in Fischer 344 rats. In *Renal Effects of Petroleum Hydrocarbons. Advances in Modern Environmental Toxicology,* ed. M. A. Mehlman, G. P. Hemstreet, J. J. Thorpe, N. K. Weaver, 7:65–71. New Jersey: Princeton Sci.
133. Natl. Toxicol. Progr. 1985. Carcinogenesis bioassay of Isophorone in F344/N rats and B6C3F1 mice (gavage study). *NTP Tech. Rep. No. 291*
134. Natl. Toxicol. Progr. 1988. Carcinogenesis bioassay of *d*-limonene in F344/N rats and B6C3F1 mice (gavage study). *NTP Tech. Rep. No. 347.*
134a. Borghoff, S., Short, B., Swenberg, J. A. 1990. Biochemical mechanisms and pathobiology of $\alpha_{2\mu}$-globulin nephropathy. *Annu. Rev. Pharmacol. Toxicol.* 30:349–67
135. Swenberg, J. A., Kerns, W. D., Mitchell, R. I., Gralla, E. J., Pavkov, K. L. 1980. Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor. *Cancer Res.* 40:3398–402
136. Lee, K. P., Trochimowicz, H. J., Reinhardt, C. F. 1985. Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. *Toxicol. Appl. Pharmacol.* 79:179–82
137. Mauderly, J. L., Jones, R. K., McClellan, R. O., Henderson, R. F., Griffith, W. C. 1986. Carcinogenicity of diesel exhaust inhaled chronically by rats. In *Carcinogenic and Mutagenic Effects of Diesel Exhaust,* ed. N. Ishinishi, A. Koizumi, R. O. McClellan, W. Stober, pp. 397–409. Amsterdam: Elsevier. 537 pp.
138. Vostal, J. 1986. Factors limiting the evidence for chemical carcinogenicity of diesel emissions in long-term inhalation experiments. See Ref. 137, pp. 381–96
139. Morrow, P. E. 1988. Possible mechanisms to explain dust overloading of the lungs. *Fundam. Appl. Toxicol.* 10:369–84
140. Wakabayashi, K., Iakagi, T., Fujimoto, Y., Fukuda, Y. 1978. Induction by degraded carrageenan of colorectal tumors in rats. *Cancer Lett.* 4:171–76
141. Oohashi, Y., Ishioka, T., Wakabaya, K., Kuwabara, N. 1981. A study on carcinogenesis induced by degraded carrageenan arising from squamous metaplasia of the rat colorectum. *Cancer Lett.* 14:262–72
142. Haber, H., Milne, J. A., Symmers, W. St. C. 1980. The skin. In *Systemic Pathology,* ed. W.St.C. Symmers, 6: 2575–604. London: Churchill-Livingstone. 2929 pp. 2nd ed.
143. Allen, C. 1953. The skin. In *Pathology,* ed. W. A. D. Anderson, pp. 1130–86. London: Henry Kimpton. 1393 pp. 2nd ed.
144. Johnson, P., Williams, R. 1987. Cirrhosis and the aetiology of hepatocellular carcinoma. *J. Hepatol.* 4:140–47
145. Edmondson, H. A., Henderson, B., Benton, B. 1976. Liver cell adenomas associated with use of oral contraceptives. *New Engl. J. Med.* 294:470–72
146. Aldinger, K., Ben-menachem, Y., Whalen, C. 1977. Focal nodular hyperplasia of the liver associated with high-dosage estrogens. *Arch. Intern. Med.* 137:357–59
147. Nissen, E. D., Kent, D. R. 1979. Role

of oral contraceptive agents in the pathogenesis of liver tumors. *J. Toxicol. Environ. Health* 5:231–54
148. Lupulescu, A. 1983. See Ref. 94, p. 137
149. Thomas, G., Williams, E. D. 1990. Evidence for and possible mechanisms of non-genotoxic carcinogenesis in the rodent thyroid. *Mutat. Res.* In press
150. Payling-Wright, G., Heard, B. E. 1980. The lung. See Ref. 142, pp. 383–409
151. IARC. 1987. See Ref. 120, pp. 106–16
152. IARC. 1987. See Ref. 76, pp. 488–93
153. Diggle, G. E. 1986. Iatrogenic neoplasia. In *Iatrogenic Diseases,* ed. P. F. d'Arcy, J. P. Griffin. Oxford: Oxford Univ. Press. 3rd ed.
154. Arden, G. P., Bywaters, E. G. L. 1978. Tissue reaction. In *Surgical Management of Juvenile Chronic Polyarthritis,* ed. G. P. Arden, B. M. Ansell, pp. 263–75. London: Academic
155. Eisenbud, E., Walter, R. M. 1975. Cancer at insulin injection site. *J. Am. Med. Assoc.* 233:985
156. Golberg, L. 1960. "Imferon". *Br. Med. J.* 1:958–60
157. IARC. 1987. See Ref. 120, pp. 297–98
158. Clemmensen, J. 1975. Phenobarbitone, liver tumors and thorotrast. *Lancet* I:37–38
159. Comm. Principal Investigators. 1978. A co-operative trial in the primary prevention of ischemic heart disease using clofibrate. *Br. Heart J.* 40:1069–118
160. Bonneterre, J., Peyrat, J. P., Demaille, A. 1985. Prolactin (PRL) and breast cancer: An Update. *Breast Diseases—Senologia* 1(1):3–26
161. IARC. 1987. See Ref. 120, pp. 299–300
162. IARC. 1979. See Ref. 69, pp. 131–33
163. Lupulescu, A. 1983. See Ref. 94, p. 130
164. Harvey, R. F., Bradshaw, M. J., Davidson, C. M., Wilkinson, S. P., Davies, P. S. 1985. Multifocal gastric carcinoid tumors, achlorhydria and hypergastrinaemia. *Lancet* I:951–53
165. Roe, F. J. C. 1989. Non-genotoxic carcinogenesis: implications for testing and extrapolation to man. *Mutagenesis* 4: 407–11

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