COMMENTARY

Preneoplastic lesions as end points in carcinogenicity testing.
II. Preneoplasia in various non-hepatic tissues

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Introduction
In addition to hepatic preneoplasia as discussed in the first part of this commentary (1), preneoplastic lesions have also been described in a number of other tissues (2). They have not been used systematically in the evaluation of carcinogenesis bioassays up to the present but should be studied in more detail and taken into consideration in the future. The following summary will not include the majority of 'dysplastic' and 'hyperplastic' lesions regarded as precancerosis in various tissues, such as the uterine cervix, skin or breast, which have been discussed in excellent reviews recently (4,5). I will rather try to cover those lesions which may be classified as 'preneoplastic' according to the definition given in part I (1).

Foci of altered cells in various tissues

Intrahepatic biliary system
It has been known for a long time that hepatotropic carcinogens frequently induce a characteristic lesion called 'cholangiofibrosis' (or 'adenofibrosis') in addition to the preneoplastic or neoplastic changes of the liver parenchyma discussed earlier (6—8). Cholangiofibrosis develops especially after short-term application of high doses of carcinogens, or in long-term studies using doses which produce considerable necrotic changes of the liver parenchyma. The pathogenesis and significance of the cholangiofibrosis for liver neoplasia has been a matter of controversy (4,8,9), but many findings suggest that the lesion is preneoplastic and may progress to cystic cholangiomas or cholangiofibromas and eventually also cholangiocarcinomas after long lag periods (10—16). Cholangiofibrosis apparently originates from the ductular epithelium via a ductular (oval) cell proliferation (12,13,17). The development of many goblet cells excessively storing neutral acid mucopolysaccharides (also called 'intestinal metaplasia') within the proliferative ducts seems to be specific for liver intoxications by hepatocarcinogenic compounds (12,18).

Pancreas
The morphogenesis of pancreatic carcinomas has been extensively studied in rodents treated with various carcinogens in the past decade (19—28). The apparent species specificity of the carcinogens used and the seeming disparity of the target cells in the different experimental models have generated considerable controversy as to the cells of origin of pancreatic carcinoma. In the azaserine-treated rat model, the earliest morphological alterations were described in acinar cells, leading to the development of nodules of atypical acinar cells (29) and eventually to acinar cell carcinomas (30). The phenotypic alterations that characterize the cells in atypical acinar cell nodules include (i) reduced cytoplasmic basophilia, (ii) reduced zymogen content and increased cytoplasmic basophilia, and (iii) nuclear abnormalities including enlargement and/or irregular shape. The early morphological changes in the hamster (19,23,24,27,28), guinea pig (21) and rat (31,32) models have been described as duct-like structures the origin of which from preexisting centroacinar and ductular cells or 'dedifferentiated' acinar cells is controversially discussed (33). Transient changes in carbohydrate metabolism with an accumulation of mucus in goblet cells, occasional storage of glycogen and increased activity of glucose-6-phosphate dehydrogenase in regions of epithelial atypia (so-called dysplastic lesions) have been described in early lesions during pancreatic carcinogenesis in hamsters (27,28).

Colon
Shamsuddin and Trump (34) reported a transient excessive accumulation of sialomucins during the early stage of experimental colon carcinogenesis induced in rats with azoxymethane. These observations in experimental animals are of particular interest, since changes in the amount and composition of mucous substances are well known from many human tumors, including colon carcinomas.

Lung
Studies on preneoplasia in the lungs of man and experimental animals have been reviewed by Gusterson (35). Detailed ultrastructural investigations comparing preneoplastic lesions in the bronchial tree of hamsters and man have demonstrated very close correlations between the preneoplastic lesions and the tumors produced in both species (36—38). When a mixture of benz[a]pyrene and ferric oxide is instilled in the trachea of Syrian hamsters a sequence of changes takes place starting with an acute reaction within 24 h to produce 'goblet cell hyperplasia' (36). With continued application of the carcinogen, the epithelium becomes hyperplastic, forming 5—10 cell layers. At this stage, the mucosa takes on the light microscopic features of squamous metaplasia, but at the ultrastructural level the cells still contain mucus in addition to tonofilaments. However, metaplastic changes are not necessarily a manifestation of early neoplasia (39). Sequential morphologic alterations in bronchial APUD cells of hamsters treated with nitrosamines have been described during tumorigenesis by Reznik-Schüller (40,41). The same author claimed that after 3 weeks treatment with N-nitrosomorpholine, lamellar bodies, similar to those normally seen in type II pneumocytes, develop in the non-ciliated Clara cells of the terminal bronchus. These cells increase in number and eventually develop into invasive tumors resembling human alveolar carcinomas.

Kidney
Renal carcinogenesis has mainly been studied in rats treated with nitrosamines (42—46). 'Proliferative tubules' lined by an irregular epithelium (47) which occasionally showed clear cells (42,48,49) were the first early lesions described. Systematic studies of the morphogenesis of epithelial kidney tumors of the rat induced by N-nitrosomorpholine revealed that at least three different types of preneoplastic tubular lesions can be distinguished, each of which appears to be the precursor of a cytologically characteristic tumor type. Thus, clear cell tubules storing
glycogen in excess regularly precede the development of clear and granular cell tumors (50). Oncocytic (distal) tubules are preaggregates of renal oncocytomas (51,52), and chromophobic and/or basophilic (proximal) tubules, which frequently store acid mucopolysaccharides, seem to be closely related to the genesis of chromophobic and basophilic renal epitheliomas (53). Different changes in the activity of a number of enzymes have recently been shown in the oncocytic and basophilic tubules (54). It is noteworthy that all of these cytoplastically different types of tubular lesions and tumors frequently develop side by side in the same kidney. As a rule, the preneoplastic tubular changes appear multicentrically and bilaterally.

**Urinary bladder**

Morphologically, preneoplasia (dysplasia) in the urothelium may be defined as a slight or moderate qualitative abnormality of basal or intermediate cells without an appreciable increase in the number of cells, i.e. without significant hyperplasia in a flat lesion (55). In rats, mice and dogs treated with chemical carcinogens, a focal loss in the activity of alkaline phosphatase has been demonstrated in non-hyperplastic and hyperplastic lesions of the bladder epithelium by a number of authors (56–59). Increased activities of non-specific esterases and β-glucuronidase have also been described in hyperplastic bladder lesions of these species, but comparable enzymatic changes did not occur in bladder mucosa of hamsters and guinea pigs treated with N-butyl-N-(4-hydroxybutyl)nitrosamine (80). In rats, the deficient activity in alkaline phosphatase persisted throughout the preneoplastic phase, and a similar loss in enzyme activity was also found in many, albeit not all papillomas and transitional cell carcinomas (59). However, similar losses of alkaline phosphatase activity are seen in reversible regenerative hyperplasias of the bladder induced by various methods, such as intraperitoneal cyclophosphamide injection or ulceration of the bladder by freezing (55). Alkaline phosphatase activity returns to normal levels as the hyperplastic changes resolve under these conditions. Studies of β-glucuronidase demonstrate that, as for alkaline phosphatase, the levels decrease in reversible regenerative hyperplasias as well as in transitional cell carcinomas (55). Vanderlaan et al. (61) described that early bladder lesions induced in rat with N-butyl-N-(4-hydroxybutyl)nitrosamine were usually negative for γ- GT, but showed an increased activity of NADH diaphorase.

**Brain**

A transient focal accumulation of mucopolysaccharides as demonstrated by staining with alcian blue or by the iron binding method has been observed during the development of gliomas induced in rat with nitrogenous derivatives (62,63). These lesions developed after long lag periods. In small gliomas, a high amount of the mucopolysaccharides was also encountered. However, with increasing tumor size a gradual reduction of the polysaccharides initially stored in excess was frequently seen (63).

**Concluding remarks**

The preneoplastic focal lesions identified by cytomorphological and cytochemical methods in a number of tissues, especially in the liver, may well be used as end points in carcinogenicity testing. However, many possible pitfalls are evident, and no experimental model is available which might serve as a general test system for the carcinogenic potential of chemicals. Organotropic and cytotoxic effects of the chemicals apparently play a crucial role in the production of preneoplastic as well as neoplastic lesions. Thus, preneoplastic lesions in a certain type of tissue do not necessarily predict a similar effect in any other tissue. Moreover, a certain type of preneoplastic lesion may not predict all types of tumors potentially developing in this tissue. Nevertheless, the detection of preneoplastic lesions in whole animal studies seems to be more reliable for risk assessment than any known in vitro test. The persistence of specific phenotypic cellular change induced by a chemical, but independent of its further action after withdrawal, is an important indicator of preneoplastic lesions. The cells composing these lesions are usually characterized by metabolic aberrations which are expressed in changes of the activity of various enzymes, and frequently also in a transient excessive storage of polysaccharides (glycogen, mucopolysaccharides) or other functional abnormalities. However, the use of sophisticated histochemical methods is usually not a prerequisite for the detection of the lesions since most of them are also readily visible in conventional hematoxylin and eosin sections. Studies in some tissues, especially the liver parenchyma, suggest that an ordered sequence of metabolic and morphologic cellular changes, which lead to a progression-linked phenotypic instability, is followed during the process of carcinogenesis. Foci of altered hepatocytes have been shown to predict hepatocarcinogenicity of the compounds tested. However, a lack of such lesions does not exclude carcinogenicity. Simple experimental systems of the stop type are preferable to complex models, although such simple systems may not fulfill the requirements for a 'short-term test'. The results obtained in the complex models have to be interpreted very cautiously, especially when known carcinogenic chemicals are administered in addition to the test compound. The old discussion on the possibility of separating clearly 'initiating' from 'promoting' properties of the test chemicals following operational definitions remains controversial at the level of preneoplasia.

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**References**


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