gest that, following treatment of carpet, only a very small fraction of the applied pyrethroid can indeed be regarded as potentially bioavailable by inhalation (max. 0.2% of the active ingredient applied to the carpet). Therefore, assessment of health hazards in the indoor environment based solely on "vacuum cleaner" sampling (as used by analytical chemists to search for indoor contaminants) rather than examination of the actual airborne concentration, including other relevant airborne materials, is prone to tremendous errors and misjudgments.

These examples demonstrate that new regulatory requirements, *e.g.,* the EU biocide directive, and public perception and concern may trigger complex and demanding toxicological examinations and standardization of such complex studies with regard to the generation of test atmospheres, mode and duration of exposure, and selection of adequate toxicological endpoints, as a basic prerequisite for state-of-the-art hazard identification and risk assessment.

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Adverse effects of environmental exposures may occur not only in the individuals directly exposed, but also in their progeny

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Individual differences in the risk of cancer are ultimately determined by the interaction between the environment, that is the complex of factors that are not part of the genetic patrimony at the time of conception, and the genotype. Beginning prenatally, humans are exposed throughout life to a variety of environmental agents, among them many carcinogens and mutagens. Adverse effects of such exposure may occur not only in the individuals directly exposed, but also in their progeny.

Prenatal events may contribute to increase the cancer burden following either: i) exposure to a noxious agent during pregnancy with consequent direct exposure of fetal cells *in utero* (transplacental carcinogenesis), or ii) exposure of one or both parents to a carcinogen/mutagen before conception, resulting in a possible alteration of the germ cells (transgenerational carcinogenesis).

There is experimental evidence of a transplacental carcinogenic effect for a large number and variety of chemical carcinogens. Evidence in humans is instead limited to exposure during pregnancy to diethyisfilbestrol (DES) and X-rays. Studies in rodents, however, cover the entire lifespan of the progeny and the increase in cancer frequency is generally observed in adult or late life. Observations in humans are almost exclusively confined to the occurrence of Cancer in childhood.

Even if many experimental studies remain open to criticism, particularly due to the relatively small number of animals used and the consequent lack of statistical power of the data, there is convincing evidence for a transgenerational effect in laboratory animals of a few chemical Carcinogens and of internal and external exposure to radiation. The epidemiological data in humans are mostly derived from the investigation of paternal occupational exposures to chemicals, chemical mixtures or radiation and, although suggestive of a transgenerational effect, they remain Controversial. Observations in humans, again, are limited to the occurrence of childhood cancer.

The hypothesis has been proposed that certain environmental exposures of parents before conception may cause alterations of the germ cells which affect the susceptibility of the progeny to cancer. Most likely, different mechanisms underlie the high incidence of tumors appearing at an early age in certain familial syndromes, and the surfacing of an increased predisposition to cancer revealed by a relatively modest increase in tumor incidence late in life. A better understanding of pre- and postnatal gene-environment interactions in determining cancer risks would have important implications for public health, as it could considerably improve the efficacy of primary prevention.

Experimental adenocarcinoma of the esophagus: Implications in the sequence Barrett's esophagus-adenocarcinoma

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No suitable models of esophageal carcinogenesis were available until the discovery by Duckrey *et al.* that N-methyl-N-nitrosoaniline given orally to rats resulted in a high incidence of squamous cell carcinomas of the esophagus. Since then, most of the experimentally induced squamous cell carcinomas of the esophagus have been due to the exposure of rats to several nitrosamines by various routes of administration.

In recent years, the incidence rates for adenocarcinoma of the esophagus have risen rapidly in Western countries, especially among white males. Most of these tumors arise from areas of columnar-lined esophagus (Barrett's esophagus), a condition clearly associated with a chronic gastroesophageal reflux of acid and duodenal-content secretions. These epidemiological observations prompted us

to study the possible role of duodenal-content reflux esophagitis in a model of esophageal carcinogenesis with 2,6-dimethylnitrosomorpholine (2,6-DMNM) in which squamous cell carcinoma was the histological type usually induced.

Materials and methods

The induction of adenocarcinomas of the esophagus was accomplished in Sprague-Dawley rats under the combined influence of chronic esophagitis plus the carcinogenic effect of 2,6-DMNM. Chronic reflux esophagitis was produced by means of an esophagojejunostomy. This procedure, which diverts the biliary and pancreatic juice into the esophagus, significantly increased the number of animals with esophageal carcinomas (co-carcinogenic effect) after the chronic subcutaneous administration of 2,6-DMNM.

Results

Most strikingly, this model resulted for the first time in the induction of a significant number of carcinomas with glandular differentiation. It is of interest that reflux esophagitis of long duration (20-30 weeks), without administration of carcinogen, induced the development of foci of glandular metaplasia in the esophagus of rats. This finding suggests that glandular metaplasia may represent a morphological substrate from which the adenocarcinomas originate, because only squamous cell carcinomas were observed when 2,6-DMNM was given to rats that did not have esophagojejunostomy.

Discussion

In a subsequent study which aimed to determine which fraction of the duodenal-content reflux, pancreatic or biliary, contributed to the development of esophageal adenocarcinomas, it was found that adenocarcinomas developed only in those 2,6-DMNM-treated rats exposed to reflux of pancreatic secretions, either alone or in combination with bile. Adenocarcinomas were not observed in the group of carcinogen-treated rats exposed to bile reflux alone. Recent observations have demonstrated that duodenal-content reflux of longer duration (40-50 weeks) *per* se in the absence of exogenous carcinogens may induce the development of esophageal carcinomas, especially adenosquamous carcinomas. It was also common to observe the appearance of multiple foci of glandular metaplasia in the squamous epithelium. All these findings support the following hypothesis for the development of carcinomas arising in rat esophagus: pancreatic reflux injures the squamous epithelium, and unknown factors contained in this secretion promote a double differentiation capability in the proliferating stem cells of the basal layer of the squamous epithelium. In some cases foci of glandular metaplasia may also arise. Exogenous or endogenous carcinogens present in the biliopancreatic secretion, acting upon these stem cells, then induce the development of adenocarcinomas or adenosquamous carcinomas.

All these findings support the role of duodenal-content secretions on the process of mucus differentiation in the squamous epithelium. These observations may help to understand some aspects of the pathogenesis of Barrett's esophagus. In addition, the effect of biliopancreatic secretions on the sequence reflux esophagitis-mucus differentiation-adenocarcinoma/adenosquamous carcinoma in the rat model support the role of duodenal-content secretions in the process of malignant transformation in Barrett's esophagus as has recently been suggested.

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Biological investigations of environmental and occupational compounds using an alternative *in vitro* concept

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Assessment of cytotoxicity of inhalable substances such as gaseous or particulate compounds and complex mixtures have traditionally involved animal experiments. Difficulties in the calculation of human risk from animal data and the high number of relevant substances raise the question of effective alternative test systems to analyze the biological effects of airborne matter. *In vitro* systems offer the unique possibility to analyze the cellular reactions dependent on substance, concentration and time and to compare the data of several substances in the same system. The use of human cells, in particular, reduces difficulties in interpreting and extrapolating animal data to the human situation.

New cultivation and exposure techniques in the field of *in vitro* toxicology also enhance the efficiency of such cellular studies, as demonstrated by two experimental setups which allow direct exposure of cells from the respiratory tract at the air/liquid interface. The basic feature in both cases is the cultivation of the cells on porous transwell membranes, which are permeable for the culture medium.

The first system, called "CULTEX", is based on an intermittent medium supply of the cells. We have developed this alternative cultivation system, which provides a flexible and reproducible experimental protocol, to cultivate and expose cells to airborne material at the air/liquid interface, The method as well as the culture chamber are already patented. The medium will be pumped into four special modular culture units, each housing three transwells, through the transwell membrane to support the cells. At certain time intervals, the medium is be removed completely and the cells can be maintained and exposed at the air/liquid interface until the next medium supply without loss of viability for up to 72 h. Both the wells and the individual modules are connected via a network of glass tubes and hoses. Starting from a central medium supply, the medium is directed by a peristaltic pump via distribution nozzles to the relevant