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New Trends in Genetic Risk Assessment

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Part VI

Test Selection and Risk Assessment

Introduction

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During the last 20 or so years the research of many investigators has been based on the tacit assumption that all cancers find their roots in genotoxic damage by exogenous environmental agents. The first three of the following contributions concern the choice of short-term approaches or tests for the prediction of genotoxic carcinogenicity. In other words, they all concern the detection of genetic risk by chemical or other agents – either natural or xenobiotic – to the genetic component of individual cells, and are not concerned with genetic damage to future generations of whole animals. Because one should not brush aside examples of carcinogenesis following exposure to agents that give convincingly negative results in a wide spectrum of tests for genotoxicity, it seems indeed desirable to discuss here the relative contributions of genotoxic and non-genotoxic mechanisms to the total human cancer burden.

Investigators dedicated to the belief that all carcinogenicity results primarily from genotoxic damage do have various grounds for their opinion. These include possible inadequacy or insensitivity of the methods used for detecting genotoxicity. Belief in this explanation has spurred on research aimed at developing ever more ingenious and/or more sensitive short-term tests for genotoxicity. It is particularly the problems posed by the existence of a multiplicity of test methods – some well-validated and

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some not – and by the fact that the application of batteries of such tests to the same test substance not infrequently engenders a spectrum of findings ranging from completely negative to convincingly positive results that are addressed in the following contributions. An alternative argument is that if a non-genotoxin causes cancer it does so by *promoting* the selective multiplication of cells previously damaged by exposure to genotoxins such that they give rise to localized neoplasms. In the absence of any reliable way of detecting isolated genetically-damaged cells within tissues, this hypothesis lacks any firm foundation but cannot be disproved. There is, of course, no lack of genotoxic factors in the background environment. The DNA of all living creatures is constantly bombarded by cosmic ionizing radiation. Nor is there any way of escaping exposure to terrestrial sources of ionizing radiation, such as radon which constantly seeps out of rocks (e.g. granite) which contain traces of uranium. During normal physiological processes involved in the metabolism of ordinary nutrients, electrophilic metabolites capable of damaging cellular DNA are constantly being produced and, apart from this, it would be virtually impossible to devise an appetizing diet for humans which did not include a wide variety of naturally-occurring genotoxins or substances which can be metabolized to genotoxins. Among them are potent genotoxic carcinogens such as aflatoxin derived from moulds. Worse still, procedures designed to reduce the risk of food poisoning from bacterial and fungal toxins (e.g., the addition of nitrite or anti-oxidants and cooking) may at the same time introduce genotoxic activity into food.

Given that genotoxins abound in the natural environment, is there any point in trying to reduce exposure to DNA-damaging activity by striving to distinguish between substances which are and those which are not genotoxic? Conventional practice, if not wisdom, dictates that genotoxic xenobiotic agents should not be added to food and, as far as possible, should not be used as drugs. However, whether compliance with this practice has any substantial effect on human cancer risk is dubious and certainly not proven.

During the past few years interest has grown in mechanisms involved in non-genotoxic carcinogenicity. In laboratory animals a wide variety of quite different mechanisms have been identified, and only very few of these fit the two-stage carcinogenesis paradigm which casts such agents in the role of tumour-promoters capable of enhancing cancer risk by selectively stimulating the multiplication of cells previously damaged by genotoxins. The biggest single determinant of cancer risk in laboratory rodents not deliberately exposed to genotoxic agents is how much they eat. Caged laboratory rodents given free access to food throughout the 24 hours of each day tend to become obese, to develop endocrine

disturbances, to age prematurely and die early of a variety of non-neoplastic diseases and to develop benign and malignant neoplasms of a wide variety of both endocrine and non-endocrine tissues. By simply restricting intake to about 80% of the amount consumed by *ad-libitum*-fed animals the incidence of all these problems can be highly significantly reduced. This observation is simply not plausibly explained by supposing that the differences observed are attributable to a 20% reduction in the intake of natural genotoxins in food. The true explanation is seemingly more complicated and involves a wide variety of mechanisms. Overnutrition-related disturbances of endocrine status are frequently seen as a prelude to enhanced incidence of mammary, pituitary and various other endocrine tumours in laboratory rats, while disturbance of mineral balance is probably implicated in the enhancement of adrenal medullary tumour development in overfed rats and in rats given high doses of relatively poorly digestible carbohydrates such as lactose and various polyols. Protease inhibitors in soya disturb cholecystokinin status in rats, and this disturbance predisposes to enlargement and neoplasia of the exocrine pancreas.

Several naturally-occurring hormones are known to be capable of predisposing to increased cancer risk both in animals and in man. These agents are not genotoxic, and the tumours that arise in response to them invariably do so against a background of *pre-existing* hyperplasia and increased cell proliferation. Frequently, a progression from hyperplasia through benign neoplasia to malignant neoplasia is easy to see. Such a sequence does not, however, fit the two-stage carcinogenesis paradigm according to which genetic damage *preceeds* cell proliferation. In other words, genotoxic events seem to occur *late* and not *early* during the course of hormonal carcinogenesis. Correction of hormonal disturbances before genotoxic events have happened usually leads not only to disappearance of the cell-proliferative changes but also to that of any enhanced risk of cancer development.

A classical example of non-genotoxic, hormonal carcinogenesis is provided by the results of experiments in which both ovaries are removed from an otherwise normal rodent and then one of the excised ovaries is implanted into the spleen. Gonadotropic hormone produced in the pituitary stimulates the intrasplenic ovary to produce oestrogen. However the oestrogen which it produces passes straight to the liver via the splenic vein and is there broken down. As a consequence little or no oestrogen reaches the pituitary where it normally acts to inhibit the overproduction of gonadotropic hormone as part of a negative feed-back homeostatic control mechanism. The eventual outcome of this surgically-manipulated interference with sex-hormone status is the development of pituitary

neoplasia and ovarian neoplasia along with toxic peliosis hepatitis in the overworked liver.

These observations illustrate rather clearly that prolonged stimulation of cells to proliferate and excrete hormones is associated with increased risk of development of neoplasia. Why should this be? A possible explanation is that cell division itself is not completely free from risk of error capable of resulting in genetic deviation (e.g. at the level of chromosomal aberration). Alternatively, the increased metabolic activity associated with active cell proliferation and with increased secretory activity may be associated with increased endogenous production of electrophiles capable of damaging DNA, and even if the vast majority of the damage produced by endogenous electrophiles is completely and accurately repaired, there will be a slow build-up of unrepaired or inaccurately repaired DNA damage which eventually leads to malignancy. *Whatever the mechanisms involved, it has to be accepted that carcinogenesis by non-genotoxic substances, including endogenously produced natural hormones, is a reality.*

It is against the distinction between genotoxic and non-genotoxic mechanisms that one needs to consider the choice of methods to include in a battery of tests for the assessment of genotoxic carcinogenicity.