review article

Short term screening tests for carcinogens

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There are now short term tests with a high predictive value for mammalian carcinogens. Many of them are based on the ability to detect damage to DNA in bacteria or mammalian cells after metabolic activation by microsomal enzymes. Their introduction will enable provisional safety assessments to be made for the many thousands of industrial and environmental chemicals for which long-term animal testing cannot at present be considered.

IT has been estimated[1] that if one could totally abolish human cancer it would add a mere two years to the average lifespan. Most cancer sufferers are past retiring age so that industrial production would be little affected by the abolition of cancer The fight against cancer must instead be justified in terms of the cost of hospital services and of basic humanity; treatment of cancer, even when it is successful, is a miserable process. When it fails, as it so often does, one feels guilty of a double offence, not only the loss of the patient, but the imposition of heroic measures that themselves may cause considerable physical and mental suffering.

The International Agency for Research on Cancer holds it as a rule of thumb that around 80% of cancer has an environmental cause[2,3], others would give a higher figure[4]. The evidence is indirect, being based on differences in tumour incidence between genetically similar populations in different environments[2,3,5]. Even if this estimate is only approximately correct it leads ineluctably to the conclusion that a substantial proportion of cancers, possibly a majority, are in principle preventable. In past decades those responsible for the disbursement of cancer research funds have tended either to look for a breakthrough in the area of curative treatments or to make a long term investment in basic biology in an attempt to understand the disease (or more properly diseases since "cancer" is but a general term for hundreds of different malignant conditions). Recently, however, these two essential approaches have been complemented by a third, the search for the specific environmental factors involved in carcinogenesis.

The nature of these environmental factors is not known in detail, but it seems likely that many of them are man-made or natural chemicals. Even factors such as diet or stress may act indirectly by altering the metabolism of chemicals in the gut or in the body itself. Of course, identification of environmental carcinogens does not necessarily lead to their removal but it does open the way to control so that the risk that they present is no more than is necessary when weighed against any benefits that they may give.

The most direct method of identifying environmental carcinogens for man is based on population studies, but unfortunately it is expensive and seems to have rather low resolving power. Only a handful of chemicals are known to be carcinogenic to man and most of these have been detected following the study of workers occupationally exposed to chemicals capable of giving rise to specific and rather rare neoplasms. The classic case is soot which has been known for 200 years to produce scrotal cancer in young chimney sweeps[6]. More recent examples are 2-naphthylamine, vinyl chloride and asbestos which produce, respectively, rare cancers of the bladder, angiosarcomas of the liver, and mesotheliomas of the lung cavity. The problems involved in identifying two populations differing only in their exposure to one chemical are formidable and are further compounded if the chemical gives rise not to specific and otherwise rare tumours, but to a variety of common cancers. Population studies are thus likely to be of limited value in identifying environmental (as distinct from occupational) carcinogens but they will be indispensable in providing the basis for risk evaluation, particularly where dose-response data can be obtained.

The alternative is to screen chemicals to which man is exposed. The generally accepted method of doing this is to carry out long term carcinogenicity tests with laboratory mammals. Not only are those tests very demanding of resources but any extension of animal testing on such a wide scale would be vigorously opposed by a number of animal welfare lobbies. In practice, it is inconceivable that resources could be made available (either men, money or mice) on the necessary scale to screen all the tens of thousands of substances to which humans are exposed. Of necessity, therefore, testing with whole mammals will be restricted to certain groups of suspect substances, for example those suspect but already in use on a large scale, or those substances which it is proposed to administer on a large scale, as food additives or cosmetics, for instance.

If one is to screen for carcinogenic chemicals, therefore, one must use short term tests with a high predictive value. I propose to review a number of possible systems which have been suggested in recent years. As will become apparent, many of them are in fact systems for the detection of agents causing damage to DNA. Damage to DNA leading to heritable changes may be important to man not only because of carcinogenicity but because it may cause hereditary disease[7-9]. Moreover, DNA damage may conceivably be involved in ageing and diseases associated with ageing[1]. I take it as self-evident that any agent likely to damage the DNA of man, whether in somatic or germ cells, is potentially hazardous.

Screening systems

The induction of cancer is but one aspect of long term toxicity and for the evaluation of such hazards a three-tier

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approach has been proposed[8,11]. The first tier would consist of simple short term sub-mammalian tests with a high predictive value for the human effect ultimately of interest. As many substances as possible should be screened with these tests. Second-tier tests would be both short and long term, on mammals. Only selected, high priority substances would be screened by these tests in addition to the first-tier tests. Tests in the third tier are designed not to detect toxic agents but to evaluate as quantitatively as possible the hazards to man from agents shown to be potentially toxic. Only substances whose use or presence seems inescapable would be subject to the third tier of evaluation, the object of which would be to make a risk–benefit assessment and institute appropriate regulatory action. Tests in successive tiers show in principle increasing relevance to man but this is often accompanied by decreasing sensitivity and practicability.

Not all of the sub-mammalian tests depend upon the postulated electrophilic nature of the active forms of carcinogens and in particular on their ability to react with DNA. Williams and Rabin[12], for example, have proposed that substances might be screened using a test based on membrane–polysome association. They found that a number of carcinogens caused degranulation of rough endoplasmic reticulum (microsomal membranes) from male rat liver. This test has been further developed by Purchase and Lefèvre[13] who have measured the loss of radioactive RNA from rough endoplasmic reticulum. Preliminary results (D. Anderson *et al.*, unpublished) with a large number of carcinogens and non-carcinogens indicate that the method predicts the activity of arylamines rather well (85% correct) although it is less successful with polycyclic hydrocarbons and direct acting alkylating agents.

The necessity for metabolic activation of many carcinogens by microsomal enzymes prompted the suggestion of McPherson *et al.*[14] that the specific *in vitro* enhancement of biphenyl 2-hydroxylation activity in rat liver microsome preparation might be used as a screening test. They found that of eight known carcinogens, all caused an increase of around 100% in such activity, four compounds whose carcinogenicity is in doubt gave lower but significant increases, and eleven non-carcinogenic compounds gave no significant increase. This test system, like that of degranulation of ribosomes, is obviously promising and in need of a much more exhaustive validation on a scale similar to that used with some other systems.

Metabolic activation

There is a widespread belief among cancer workers that DNA damage is involved in the induction of cancer. That is the basis for the supposition that carcinogens might be detected by the consequences of DNA damage in simple systems. Two recent developments have enabled this possibility to be realised. First, it has become clear that many carcinogens are the products of metabolism of inactive chemicals by mixed function oxidases in the animal[15], and that preparations of liver microsomes can be used *in vitro* to carry out this metabolic activation[15–18]. Second, ultra-sensitive bacterial systems, usually involving strains deficient in DNA repair, have been developed for the detection and characterisation of agents causing damage to DNA[19–22].

The first published work in which the mutagenic activity of metabolites was detected after metabolic activation of carcinogens was by Malling[17]. The methodology of his quantitative liquid assay system has been recently described[23]. Later, Ames *et al.*[24] showed that microsomes could be added to the semi-solid agar overlay in a plate test, a procedure that is in some ways rather better for routine screening although it fails with a few compounds, for example, dimethylnitrosamine, possibly because the agar interferes with the diffusion of short-lived active metabolites.

Bacteria deficient in repair of DNA are killed more easily by DNA-damaging agents than are wild type bacteria, and this is the basis for several simple tests. Bacteria deficient in excision repair have been used[25] but these are sensitive only to certain types of DNA damage. Much more useful have been bacteria lacking DNA polymerase I (Pol^-)[26], or deficient in genetic recombination (Rec^-)[27]. These tests are usually conducted on the surface of agar plates but are also amenable to rather more quantitative procedures with liquid-phase treatment[28,29].

Another way of revealing the existence of DNA damage is to look for the repair that it usually initiates and this is the basis of a very useful test developed by Stich and his colleagues. It depends on estimating the amount of DNA synthesis involved in repair by measuring autoradiographically the uptake of tritiated thymine during the period immediately following exposure to the test chemical. The method has the advantage that it can be used with cultured human skin fibroblasts. To prevent normal DNA synthesis the cells are kept in an arginine deficient medium for 3 d before exposure.

In a report on 64 substances tested, Han and Stich[30] found that all directly acting carcinogens elicited unscheduled DNA synthesis whereas no repair synthesis was observed after treatment with 16 non-carcinogens. Most carcinogens known to need metabolic activation gave negative results although a few were active after prolonged exposure to high concentrations. More recent results[31] indicate that metabolic activation systems can be incorporated in this assay and make possible the detection of pro-carcinogens.

Mutation induction

Perhaps the most sensitive assay for DNA damage is the induction of mutations in bacteria, particularly if the bacterial strain carries a mutation rendering it unable to excise damage from DNA (Uvr^-). Excision-proficient strains should always be included in any assay, however, because certain agents able to cross-link DNA are only mutagenic in such strains[32]; presumably the mutational event occurs as an error during excision-initiated repair. Reversion to prototrophy is generally regarded as the most sensitive type of assay and the methodology has recently been reviewed[33,34]. *Escherichia coli* WP2 is a tryptophan-requiring strain that responds to mutagens causing base-pair substitution mutations at both adenine : thymine and guanine : cytosine sites.

A more complete set of tester strains has been developed in *Salmonella typhimurium* by Ames and collaborators[35]. Individual strains respond to base-pair substitution mutagens or to compounds causing various types of frameshift. The permeability of these *Salmonella* strains to some chemicals has been increased by the incorporation of a cell wall mutation ("deep rough")[36]. From recent data one can calculate that these strains are capable of detecting mutagenic activity of between 61%[37] and 90% (D. Anderson, unpublished) of known carcinogens. In an attempt to detect the "false negatives" obtained with the deep rough strains, Ames's group developed a fourth generation set of strains containing the drug resistance plasmid pKM101[37]. As suggested by MacPhee certain plasmids confer a mutator activity on their host cell which becomes more sensitive to many mutagens and carcinogens[38]. The ability of these plasmid-containing strains to detect carcinogens as mutagens is impressive (see below). A word of caution is in order, however, since although the mechanism by which the plasmids act is still unknown, it is clear that they convert into mutations damage which would not be mutagenic in a normal cell. They may even act as amplifying systems and produce mutations at sites where no damage exists. The value of such strains lies in the correlation they show with carcinogenicity but there is at least a theoretical possibility of real "false positives".