

# review article

## Short term screening tests for carcinogens

Bryn A. Bridges\*

*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<sup>1</sup> 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<sup>2,3</sup>; others would give a higher figure<sup>4</sup>. The evidence is indirect, being based on differences in tumour incidence between genetically similar populations in different environments<sup>2,3,5</sup>. 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<sup>6</sup>. 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<sup>7-9</sup>. Moreover, DNA damage may conceivably be involved in ageing and diseases associated with ageing<sup>1</sup>. 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

\* Address: MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9QG, Sussex, UK

approach has been proposed<sup>10,11</sup>. 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<sup>12</sup>, 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<sup>13</sup> 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.*<sup>14</sup> 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<sup>15</sup>, and that preparations of liver microsomes can be used *in vitro* to carry out this metabolic activation<sup>16-18</sup>. 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<sup>19-22</sup>.

The first published work in which the mutagenic activity of metabolites was detected after metabolic activation of carcinogens was by Malling<sup>17</sup>. The methodology of his quantitative liquid assay system has been recently described<sup>23</sup>. Later, Ames *et al.*<sup>24</sup> 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<sup>25</sup> but these are sensitive only to certain types of DNA damage. Much more useful have been bacteria lacking DNA polymerase I (Pol<sup>I</sup>)<sup>26</sup>, or deficient in genetic recombination (Rec<sup>-</sup>)<sup>27</sup>. These tests are usually conducted on the surface of agar plates but are also amenable to rather more quantitative procedures with liquid-phase treatment<sup>28,29</sup>.

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<sup>30</sup> 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<sup>31</sup> 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<sup>-</sup>). 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<sup>32</sup>; 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<sup>33,34</sup>. *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<sup>35</sup>. 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")<sup>36</sup>. From recent data one can calculate that these strains are capable of detecting mutagenic activity of between 61%<sup>37</sup> 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<sup>38</sup>. As suggested by MacPhee certain plasmids confer a mutator activity on their host cell which becomes more sensitive to many mutagens and carcinogens<sup>39</sup>. 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".

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Group of compounds

Animal carcinogenicity and bacterial mutagenicity with and without metabolic activation (from McCann *et al.*<sup>13</sup>)

|                                         | Carcinogens detected as bacterial mutagens | Non-carcinogens not mutagenic to bacteria | Compounds of uncertain carcinogenicity detected as mutagens |
|-----------------------------------------|--------------------------------------------|-------------------------------------------|-------------------------------------------------------------|
| A Amines etc.                           | 23/25                                      | 10/12                                     | 5/7                                                         |
| B Alkaloids, etc.                       | 17/20                                      | 1/3                                       | 1/1                                                         |
| C Polycyclic aromatics                  | 26/27                                      | 7/9                                       | 1/1                                                         |
| D Esters, epoxides, carbamates, etc.    | 13/18                                      | 5/9                                       | 0/1                                                         |
| E Nitro aromatics and heterocycles      | 28/28                                      | 1/4                                       | 0/2                                                         |
| F Miscellaneous organics                | 1/6                                        | 13/13                                     | 0/1                                                         |
| G Nitrosamines                          | 20/21                                      | 2/2                                       | 1/1                                                         |
| H Fungal toxins and antibiotics         | 8/9                                        | 5/5                                       | -                                                           |
| I Mixtures (cigarette smoke condensate) | 1/1                                        | -                                         | -                                                           |
| J Miscellaneous heterocycles            | 1/4                                        | 7/7                                       | -                                                           |
| K Miscellaneous nitrogen compounds      | 7/9                                        | 2/4                                       | -                                                           |
| L Azo dyes and diazo compounds          | 11/11                                      | 2/3                                       | 3/3                                                         |
| M Common laboratory biochemicals        | -                                          | 46/46                                     | -                                                           |
| Total                                   | 157/178                                    | 101/117                                   | 11/17                                                       |

tion is only as good as the confidence one has in the reliability of both parameters. Whereas positive and negative mutagenicity results can be both unambiguous and reproducible, the same is not true of carcinogenicity results where, as will be discussed below, there are several factors which could result in a failure to detect relatively weak carcinogens. As discussed by McCann and Ames<sup>14</sup>, there is good reason to believe that many of the "false" positive chemicals will eventually be shown to be carcinogenic. This has already happened with the food additive furoyl furamide which had been used for many years in Japan and had given negative results in two carcinogenicity trials<sup>15</sup>. After positive results had been obtained in *Bacillus subtilis* and *E. coli* systems for detecting DNA damage, it was re-examined and shown to produce a low but significant yield for tumours when given to foetal and young mice<sup>16</sup>. There is also the real possibility that some of the "false" negatives are genuine, that metabolism *in vivo* is different from that with isolated microsome preparations. Only further studies in depth can resolve this.

It is worth analysing the data of McCann *et al.* further to see whether there is any particular type of mutational event (as detected by the *Salmonella*) that is correlated with carcinogenicity. It has been postulated<sup>17</sup> that carcinogenicity is associated with the ability to produce specific types of frameshift mutation. This hypothesis does not hold up in any general application. As can be seen from Table 2, whereas most members of some groups of carcinogens (for example, aromatic amines, polycyclic aromatics and nitroaromatics) gave rise to both frameshifts and base-pair substitutions, others (for example, esters, epoxides and carbamates, nitrosamines, miscellaneous nitrogen compounds) gave rise exclusively to base-pair substitutions. There was no group that gave rise exclusively to frameshifts. Taken together, 45.2% of mutagenic carcinogens gave rise solely to base-pair substitutions, 14.8% solely to frameshifts, and 40% gave rise to both.

Rosenkranz (cited in ref. 62) using a Pol<sup>-</sup> strain of *E. coli* together with the *Salmonella* set without plasmids, has obtained results as encouraging as those of McCann *et al.* with the plasmid-containing salmonellas. Of about 100 compounds tested, 85% of the known carcinogens were detected (91% of direct acting carcinogens, 72% of procarcinogens). The proportion of non-carcinogens detected as positive was rather high, 30%, but the figure is not comparable with the lower value derived from the data of McCann *et al.*<sup>13</sup> since it did not include the 46 common laboratory biochemicals tested by the latter workers, none of which was positive.

A comparison of the efficiency of various microbial systems for detecting DNA damaging agents has been carried out by Shirasu *et al.*<sup>18</sup>. They found that the hypersensitivity of repair-deficient bacteria (Rec<sup>-</sup> *B. subtilis*) was the most sensitive. Of 166 pesticides studied, 23 were posi-

tive in the Rec-assay (carried out without microsomal activation). Of the 143 negatives, none proved to be positive when tested with *E. coli* or *Salmonella* reverse mutation systems. Of the 23 positives 9 were positive in reverse mutation systems, and of these 9, 1 was not detected by the *E. coli* strains and 1 by the *Salmonella* strains. As far as base-pair substitution mutations are concerned, the non-plasmid *E. coli* strains were found to be preferable to the non-plasmid *Salmonella* strains at least with some groups such as nitrofurans. With other groups such as the organic phosphates a similar small proportion of mutagens was missed by both *S. typhimurium* and *E. coli* strains<sup>19</sup>.

The only study in which a single laboratory has compared a number of different tests for predicting carcinogenicity appears to have been carried out by the Central Toxicology Laboratory of ICI (D. Anderson *et al.*, unpublished). The preliminary results with 120 chemicals point to the value of the bacterial mutation tests when metabolic activation is incorporated. The carcinogenicity, or non-carcinogenicity was accurately predicted for 90% of the chemicals by this test. Cell transformation *in vitro* came close with 83% accuracy. Rather less accurate was degranulation of endoplasmic reticulum, 72%, and morphological changes following subcutaneous implantation, 70% correctly predicted. Sebaceous gland suppression<sup>20</sup> was good for polycyclic hydrocarbons (90%) but little better than random for other substances (52-62%). Tetrazolium reduction in mouse skin was also poor (62% overall). The authors conclude that some of these rapid tests are capable of distinguishing between carcinogens and non-carcinogens with sufficient accuracy to enable them to be used for selecting potential carcinogens. They also make the point that figures for successful prediction must be treated with

Table 2 Number of carcinogens detected as bacterial mutagens (with or without metabolic activation) classified as to type of mutation induced.

|       | Base-pair substitutions only | Frameshifts only | Both base-pair substitution and frameshift mutations |
|-------|------------------------------|------------------|------------------------------------------------------|
| A     | 1                            | 9                | 13                                                   |
| B     | 14                           | 2                | 1                                                    |
| C*    | 7                            | 5                | 14                                                   |
| D     | 13                           | 0                | 0                                                    |
| E     | 5                            | 0                | 23                                                   |
| F     | 1                            | 0                | 0                                                    |
| G*    | 19                           | 0                | 0                                                    |
| H     | 0                            | 3                | 5                                                    |
| I     | 0                            | 1                | 0                                                    |
| J     | 0                            | 1                | 0                                                    |
| K     | 7                            | 0                | 0                                                    |
| L     | 3                            | 2                | 6                                                    |
| M     | 0                            | 0                | 0                                                    |
| Total | 70                           | 23               | 62                                                   |

\* Data not available for one member.

Key for chemical groups as for Table 1. (From McCann *et al.*<sup>13</sup>)

It is possible to improve sensitivity by altering the methodology, and a modified fluctuation test has been proposed which achieves between ten and one hundredfold greater sensitivity than the conventional assay without the need for plasmid-containing strains<sup>37</sup>. There is also the possibility of developing a single tester strain that can be used to detect many different types of mutational events<sup>38</sup>. Although bacterial screening systems have proved very useful, there is still scope for further improvement.

As well as the bacterial tests that have now been extensively studied, a large number of other techniques can be used to detect DNA damaging activity and may perform a useful supplementary role, probing ambiguous or suspect results and characterising more fully the nature of the genetic damage. One may, for example, study the induction of mutations in cultured mammalian cells<sup>39-41</sup>. Mammalian cells may also be used for cytogenetic study of visible chromosome aberrations<sup>42</sup>. Recently developed staining techniques for demonstrating sister-chromatid exchanges show a greatly enhanced sensitivity<sup>43</sup> and their role in screening has been recently discussed<sup>44</sup>. Sister-chromatid exchanges may now be detected in spermatogonia<sup>45</sup> and bone marrow cells<sup>46</sup> following exposure of the whole animal to carcinogens. It is already clear, however, that although the induction of sister-chromatid exchanges is a very sensitive response to some carcinogens it occurs hardly at all with others<sup>47</sup>. Other eukaryotic mutation systems include fungi, yeasts and insects. All of these have their own advantages and disadvantages.

### Malignant transformation in cultured cells

Rather than develop a model system depending on mutation or DNA repair, others have worked towards a screening method by which transformation to the malignant condition could be brought about and detected in cell culture. The only really valid criterion for malignant transformation is the ability of a cell to produce a tumour when inoculated into an appropriate host. There are, nevertheless, several secondary criteria (discussed by Freeman and Huebner<sup>48</sup>), of which the most commonly used is the ability of cells to grow into clones in soft agar or, in the case of fibroblast cultures, to produce clones of piled-up cells when growing on a solid surface.

Most human cancers are carcinomas which are derived from epithelial cells. Relatively little work has, however, been done on the transformation *in vitro* of epithelial cells. Such cells are usually obtained from rat liver and are not easy to retain in culture in the differentiated state. Nevertheless they have been successfully transformed by 4-nitroquinoline-1-oxide<sup>49</sup>, aflatoxin B<sub>1</sub>, *N*-hydroxy-2-acetylaminofluorene, and 7,12-dimethylbenz[*a*]anthracene<sup>50</sup>, dimethylnitrosamine and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine<sup>51</sup>, and *N*-acetoxy-2-acetylaminofluorene<sup>52</sup>. Epithelial cells transformed *in vitro* usually show no altered morphology although they may grow in soft agar.

Work on fibroblasts is considerably more advanced. Fibroblasts, when transformed, give rise to sarcomas, responsible for a minority of human malignant disease. They show, nevertheless, great promise as the basis of a potential screening system for chemical carcinogens. As with bacterial mutation systems, it has often been found necessary to supplement the fibroblast's relatively poor ability to metabolise carcinogens into their active form. This has been achieved, either by cocultivation with other cells capable of carrying out metabolic activation<sup>53</sup>, or by isolating the cells from hamster embryos after treatment of the pregnant mother<sup>54</sup>.

Much of the work on transformation has been carried out with treatment of mass cultures often for long periods of time and has been subject to some criticism. Transformation has, however, been reported with short treatments followed by cloning<sup>55</sup> and this would seem to be a

better approach to adopt in future. A noteworthy feature of much work with both fibroblast and epithelial systems has been the high spontaneous rates of transformation, sometimes considerably higher than one would expect for a gene mutation. Many workers regard this as the result of the artificial environment in which the cells are cultured, and have looked (often successfully) for conditions in which the spontaneous rate is lower. Nevertheless it is likely that the rate of transformation *in vivo* is higher than has been thought and that the body is normally able to deal effectively with the aberrant cells. If this were true the role of DNA-damaging carcinogens might be seen as increasing the already high spontaneous rate and thus overloading the ability of the natural defences of the body to cope with malignant cells.

Most of the work on transformation *in vitro* has concentrated on the development of systems that can be used as models for the study of carcinogenesis (for reviews see refs 55 and 56). To the uninvolved observer, a certain amount of contradiction and inconsistency is apparent. There is, notwithstanding, an impression that this technique may soon be a valued constituent of the battery of techniques for detecting carcinogenic and DNA-damaging substances. Two recent studies have shown successful prediction of carcinogenicity almost as good as that of the bacterial mutagenicity tests<sup>57,58</sup>.

### Validation

Some of the test procedures described above are very sensitive, but how good are they as predictors of carcinogenicity? Recent reports have presented<sup>59</sup> and discussed<sup>60</sup> carcinogenicity and mutagenicity data obtained for more than 300 chemicals. All the results come from the use of *Salmonella* strains developed by Ames. Where negative mutation results had been obtained with now-obsolete strains, the test was repeated with the latest plasmid-containing strains. Testing was done both without metabolic activation and (generally) with microsomes embedded together with the bacteria in soft agar. With some nitrosamines incubation was carried out with microsomes before plating. A summary of the results is given in Table 1. Since publication, one of the non-carcinogens, 5-hydroxy 2-acetyl-amino-fluorene, has been shown to be mutagenic because of the presence of an impurity and has now been reclassified as non-mutagenic.

It can be seen that of 179 compounds whose carcinogenic effect on animals is well documented, 157 (or 87.7%) were detected as positive in the bacterial test. This level of confirmation was obtained with essentially all types of compound and was also evident for the small group of compounds for which evidence exists for carcinogenicity in man. The proportion of compounds believed to be non-carcinogenic which gave negative results in the mutagenicity tests was also high: 101 out of 117 (or 86.3%). These included 46 common biochemicals all of which were negative. Seventeen compounds were tested for which carcinogenicity data are uncertain; of these 11 were positive in the mutagenicity test.

The apparent "false" positives and negatives have been discussed elsewhere<sup>61</sup>. It is apparent that many of the latter damage DNA or cause mutations in other systems, or have mutagenic metabolites. Furthermore there is a very obvious limitation to the use of liver microsomes for activation: some chemicals may need reductive activation, or may be metabolised by the gut flora, by organs other than the liver, or by cell components other than microsomes. Indeed it is surprising that liver microsomes are as effective as they seem to be; certainly the method is capable of further improvement.

When one considers the "false" positives, that is the supposed non-carcinogens that register as mutagenic with bacteria, certain difficulties become apparent. Any correla-

some caution since they can be manipulated within wide limits by the choice of substances tested. As their substances include a large number of non-carcinogenic chemicals closely related to known carcinogens they feel that their results give a reasonably good indication of the likely value of the tests in practice.

### DNA damage and human cancer

The correlation between mutagenicity and carcinogenicity is satisfying to those who believe in the somatic mutation theory of cancer<sup>2</sup> and distressing to those who do not<sup>3</sup>. I think the correlation can be more correctly described as being between DNA damaging ability and carcinogenicity. Gene mutation is but one consequence of DNA damage; others such as chromosomal structural rearrangements, virus integration and excision, and changes in gene expression, may well be important in the carcinogenic process. Non-genetic effects are also probably involved.

One could argue that detecting DNA damage is merely a very sensitive way of detecting electrophilic reagents, and that the actual target(s) may well be in other molecules as well as or instead of DNA. This is quite possible; but there is other evidence strongly implicating DNA damage as the rate-limiting step in many carcinogenic processes.

In man, for example, mutations in five complementation groups are known to reduce or abolish the ability of cells to remove ultraviolet photoproducts from their DNA<sup>4</sup>. In all cases they enormously increase sensitivity to the carcinogenic effect of sunlight (resulting in the hereditary disease xeroderma pigmentosum). A further mutation causing the same symptoms has been shown to be associated with a deficiency in another DNA repair pathway active on newly synthesised DNA<sup>5</sup>. Another human mutation responsible for the disease ataxia telangiectasia has been shown to block repair of ionising radiation damage<sup>6</sup> and also results in proneness to develop malignant disease<sup>7</sup>. Thus, the human data strengthen our confidence in the reality of the observed correlation between DNA damaging ability and carcinogenicity. It must be emphasised, however, that even an empirical "litmus paper test", with no known theoretical basis, which gave an 80 to 90% predictiveness for carcinogenicity would be a powerful tool in the screening of chemicals for human toxicity.

### The place of tests with mammals

No single test is adequate for a first-tier (sub-mammalian) screen; most authorities agree that a battery of tests must be used as false negatives may occur with any one test. The results of these tests would be used to assign priorities for further testing using mammalian systems. At one extreme, a substance with no apparent effect on sub-mammalian systems might be given a priority so low that no further tests would be considered unless a large human population exposure were to occur or be contemplated. At the other extreme a strongly active substance might well be regarded as hazardous without further testing if the population exposed were small. If it were, say, an industrial chemical, then production workers and users ought to treat it as if it were a known toxic agent or carcinogen, at least until such time as it became possible to carry out full scale animal tests.

The greatest problem in testing for carcinogenicity or mutagenicity with mammals is the insensitivity of most of the tests. This has led, for example, to difficulties in validating microbial carcinogenicity screening systems since many of the "false" positives obtained with these are based on animal experiments that may be inadequate<sup>8</sup>. There have been, and still are, too many carcinogenicity tests with 20 or 30 animals per group.

Provided that the number of animals in each group is kept small, even a large increase in the frequency of neo-

plasms can fail to be statistically significant and enable a conclusion of "non-carcinogenic" to be drawn (see for example a recent study on the carcinogenicity of hair dyes<sup>9</sup>). As long ago as 1954, Barnes and Denz<sup>10</sup> pointed out that to detect with a probability of 0.01 an effect occurring in 1% of the animals, one would need a group of at least 455 animals. If the effect also occurred spontaneously then the number of animals per group would have to be increased manyfold. Today, notwithstanding, carcinogenicity experiments with 100 animals per group are often regarded as "good" and those with 200 animals per group are extremely rare. But although "kilomouse" experiments are theoretically attractive there may be little to be gained from them in practice. Logistical problems dictate that such experiments be phased over many weeks and involve slightly varying conditions. "Spontaneous" rates of tumour occurrence unfortunately often vary in time and place, perhaps reflecting slight differences in diet, and it is often difficult to run an adequate control group. Errors in handling are also more likely in very large experiments.

Whereas a significant reproducible positive result in a mammalian test may be taken as indicating the existence of a potential hazard for man, a negative result taken should not necessarily be taken to indicate the absence of hazard, particularly if the human population to be exposed is very large, and the number of animals in the test small. We may take some comfort where the disparity in dose between the animal and human exposure is great. This is not always so. Anaesthetic gases, for example, are given to an appreciable fraction of the population in Western society at concentrations which are not far from the lethal level. One might well feel that a negative result in a screening test with a few dozen mice would be of little value.

It can be seen that mammalian tests are not wholly appropriate for the validation of sub-mammalian tests, and it is perhaps remarkable that they should show such good agreement. Validation of one type of test against another must not blind one to the real objective, which is to predict long term toxic effects in man. There are few proven human carcinogens and most of these can be detected by both mammalian and sub-mammalian tests. In man, carcinogens tend to be recognised only when the tumour is of a rare type and there is a sufficient cluster of cases to enable association with a particular occupation to be seen by an alert clinician. Genetic effects in man are even harder to detect retrospectively. There are several examples of somatic chromosome damage in lymphocytes of persons exposed to known mutagenic and carcinogenic substances (for example, vinyl chloride<sup>11</sup>, ozone<sup>12</sup>, benzene<sup>13</sup>, toluene<sup>14</sup>, cadmium<sup>15,16</sup> and methyl mercury<sup>17</sup>). Recent evidence for the possibility of dominant lethal damage in man by vinyl chloride (P. Infante, unpublished) and anaesthetic gases<sup>18</sup> is ominous but needs closer examination.

Ultimately quantitative risk assessments must be attempted, for we must face the unpalatable fact that man will almost certainly have to be exposed to some carcinogens and mutagens whose benefits cannot be dispensed with and others which it is impracticable to eliminate from the environment. Risk evaluations at the present time almost always require information which is not available, such as the nature of the dose-effect response at low doses. Nevertheless, approaches must be found that will lead eventually to risk-benefit evaluations based less on guesswork and more upon knowledge. In the meantime the use of short term tests would enable potentially carcinogenic substances to be identified among the many thousands for which long term animal testing cannot at present be contemplated. This in turn would open the door to provisional regulatory action to minimise human exposure. Taken seriously and on a large enough scale, there is good reason to believe that this approach would ultimately result in a reduction in the incidence of chemically induced cancer.

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## articles

### Isolation and N-terminal amino acid sequence of membrane-bound human HLA-A and HLA-B antigens

John Bridgen

Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

David Snary & Michael J. Crumpton\*

National Institute for Medical Research, Mill Hill, London NW7 1AA, UK

Colin Barnstable, Peter Goodfellow & Walter F. Bodmer

Genetics Laboratory, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK

*Membrane-bound HLA-A and HLA-B antigens have been extensively purified in good yield. The sequences of the N-terminal 16 amino acids have been determined using about 1 nmol of protein eluted from polyacrylamide gel after electrophoresis in sodium dodecyl sulphate.*

The major histocompatibility region of man (HLA) occupies at least 1 to 2 recombination units on chromosome 6 and probably contains a large number of genes involved in diverse immune and possibly other functions<sup>1</sup>. The gene products that have so far been identified with this region comprise various

\*Reprint requests to M. J. Crumpton at the above address.

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