Development of a technique for the rapid screening of chemicals for carcinogenicity

A.M. COBON, L.J. LILLY & M.F. NEWTON
(introduced by F. HOBBIGER)

Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB

The conventional methods of screening chemicals for carcinogenicity, utilising rats and other mammals, are time consuming and expensive. However the realisation that carcinogenicity is highly correlated with mutagenicity (McCann, Choi, Yamasaki & Ames, 1975) has made possible the use of genetic tests (for example, the Ames' Salmonella/microsome assay) for carcinogenicity screening.

Our system (Lilly, Bahner & Magee, 1975; Newton, Bahner & Lilly, 1977) involves the scoring of chromosome aberrations in the lymphocytes of rats previously treated with the compound in question. It is cheaper and quicker than conventional long-term mammalian tests and has several advantages over microbiological and subcellular systems. These advantages may be summarised as follows:

1) The test allows for full *in vivo* metabolism of the compound by a mammal.

2) The target cells are mammalian.

3) The target cells (lymphocytes) circulate to all the metabolizing organs of the body where they may be exposed to possibly short lived metabolic products of the compounds. Such products may not survive long enough to reach more distant target tissues in the body.

4) The method permits us to distinguish between carcinogens which do and do not need metabolic activation.

5) The compound to be tested may be administered by a variety of routes, such as by injection or, more naturally, ingestion or inhalation.

6) The rat is the subject of choice for many biochemical and pharmacological investigations so that a considerable fund of knowledge concerning its metabolism and biochemistry exists.

The method we use involves exposing rats *in vivo* to the chemical under test and then allowing a suitable time lapse for it to be activated by metabolism. The rats are then sacrificed and their lymphocytes are grown *in vitro* for 48 hours. The lymphocytes are arrested in metaphase utilizing colchicine, harvested and stained for cytological examination for chromosome aberrations. Thus after a few days the effects of the compounds can be seen as compared with the months which would have to pass before induced tumours would be observable.

In addition we can culture the blood of untreated rats and add compounds to be tested *in vitro* only instead of applying them to the rats *in vivo*. In this way the action of the compound can be studied in conditions where it cannot be metabolised and it can therefore be classified as either direct acting or as needing metabolism for activation (indirect acting).

This comparison between lymphocytes exposed *in vivo* and *in vitro* can be best demonstrated by reference to some of our early work with nitrosamines (Lilly, *et al.*, 1975). If N-methyl-nitrosourea is added (0.9 mM) to cultures set up with lymphocytes from untreated animals then, because this carcinogen does not require activation *in vivo*, chromosome aberrations are induced. If however a compound which does need *in vivo* metabolism (e.g., dimethylnitrosamine) is used then there is no significant increase in the number of chromosome aberrations found in *in vitro* cultures. When dimethylnitrosamine is injected *in vivo*, so that metabolism is possible, and the lymphocytes are then cultured as usual *in vitro* chromosome aberrations are found in significantly increased numbers.

A programme using other known carcinogens is in progress to assess this system. Experiments with 1-(pyridyl-3)-3,3-dimethyltriazene (PyDT), 1-phenyl-3,3-dimethyltriazene (PDT) and N-nitrosomorpholine (NM) have shown that these compounds will give positive results in our test. It is interesting that while the two aryldiakyltriazenes, PDT and PyDT, have only questionable effects on human lymphocyte chromosomes which of course have to be exposed *in vitro*, they give very strongly positive results in our *in vivo* experiments. Similarly NM, a hepatocarcinogen which gives only weakly positive results in the Ames' Salmonella/microsome assay, can also be readily detected after rat lymphocytes have been exposed *in vivo*.

Some rather interesting experiments with nitrites have demonstrated how much quicker this test is than the long term conventional test for tumour formation. It is known (Lijinsky, Taylor, Snyder & Nettesheim, 1973) that tumours are produced in long term feeding experiments in which nitrites together with the drug aminopyrine are administered in drinking water. These are thought to be due to the formation of nitrosamines from the reaction of tertiary amines with nitrites in the acid conditions of the gut. Such experiments involve an exposure period of 30 to 50 weeks and animals are then kept till tumours develop. If quantities of nitrite and aminopyrine equivalent to one week of Lijinsky's treatment are fed to rats over only 24 h we are then able to detect a significant increase in chromosome aberrations in their lymphocytes over those found in untreated controls.

In addition to establishing the predictive value of our test we are investigating a variety of products of economic and/or of medical importance. Among these are solvents and anaesthetics which we are administering by inhalation.

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Effects of general anaesthetic agents on membrane conductance and surface potential

L. GINSBERG (introduced by F. HOBBIGER)

Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, London W1P 6DB

Many general anaesthetics alter the ion permeability of biological and artificial membranes. The mechanisms whereby such alterations in permeability are effected are unclear at present. It is known, however, that anaesthetics affect the 'fluidity' of lipid bilayers and this action has been put forward as a means whereby they might alter membrane permeability, as discussed in formal thermodynamic terms by Hill (1974). Another way in which these drugs might change membrane conductance is by altering the potential at the surface of the membrane concerned. The electrostatic potential difference across the membrane/aqueous interface has been shown to be an important factor in the control of membrane conductance both in biological systems (Chandler, Hodgkin, & Meves, 1965) and artificial preparations (Haydon & Myers, 1973). This investigation is concerned with the extent to which anaesthetic-induced changes in membrane surface potential are responsible for changes in conductance observed.

The experimental preparations were artificial planar bilayer membranes (black lipid films) formed from a solution of glycerylmonooleate (7mM) in *n*-decane and rendered conducting by the presence of the potassium carrier nonactin. The drugs studied were *n*-alkyl alcohols. Addition of ethanol to the aqueous solution bathing the membranes (100mM-KCl) produced an increase in membrane conductance. Thus, for example, ethanol (0.7M) in the bathing solution reduced the specific membrane resistance by approximately one-third. If this conductance change were solely the consequence of an alteration in membrane surface potential induced by ethanol (i.e., if ethanol is only able to bring about conductance changes by altering membrane surface potential and has no effect on other membrane properties involved in the control of permeability), then the magnitude of the underlying potential change $\Delta(\Delta\emptyset)$ may be estimated using the following expression (from Hladky & Haydon, 1973):

$$-\frac{RT}{zF}\log_e\frac{G_2(O)}{G_1(O)}=\Delta(\Delta\emptyset)$$

where $G_1(0)$ and $G_2(0)$ are the specific membrane conductances (in the limit of zero applied potential) before and after addition of ethanol, z is the valence of the current-carrying ion, R is the universal gas constant, T is the absolute temperature and F is the faraday. Applying this equation to the present data, it can be seen that the addition of ethanol (0.7M) to the aqueous phase would be expected to reduce membrane surface potential by approximately 10mv (potential inside membrane shifted in negative direction). This would be sufficient to explain the increase in conductance observed. However, it is possible to *measure* the potential change evoked at a membrane/water interface by adsorption of ethanol and compare the experimental result with that predicted on the basis of conductance data. This was achieved here by determination of compensation potential changes for spread monolayers of glycerylmonooleate at an air/water interface on addition of ethanol to the aqueous phase. Monolayer measurements of this type are directly applicable to the bilayer systems used in the conductance estimations, as demonstrated by MacDonald & Bangham (1972). At concentrations in the range used in the bilayer studies, it was found that ethanol increased the surface potential of glyceryl-monooleate monolayers by more than 100mv (potential inside membrane shifted in positive direction). Accurate estimation of the potential increase was technically difficult because of the volatility of the drug under consideration. It is clear, however, that the observed surface potential change differs markedly from that expected on the basis of the bilayer conductance studies. Indeed, the compensation potential data