Information Section

THE LEON GOLBERG MEMORIAL LECTURE

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RECENT ADVANCES IN TOXICOLOGY RELEVANT TO CARCINOGENESIS: SEVEN CAMEOS

Cameo I: A battle of giants

I first met both Leon Golberg and Frank Fairweather in 1961, shortly after my appointment as Reader in Experimental Pathology at the Chester Beatty Research Institute. The meeting, which took place in the office of the Institute's Director, Professor Alexander Haddow, before he received the royal accolade, concerned the ease with which one could produce sarcomas in rats by injecting iron dextran (Imferon) into them subcutaneously. At that time, Leon Golberg and Frank Fairweather were on the staff of Benger's Laboratories, who marketed Imferon. The atmosphere in Haddow's office was tense. Leon pointed out that the rats in Richmond's (1959) and Haddow and Horning's (1960) experiments that developed sarcomas had received overwhelmingly large doses of iron on a mg/kg/body weight basis. Unmoved by this argument, Haddow emphasized that sarcoma induction was a local phenomenon and that a 0.5-ml subcutaneous dose of iron dextran in the rat came into contact with approximately the same number of connective tissue cells as a similar sized dose in a human. The discussions reached an impasse because neither argument could be refuted on the basis of available scientific evidence.

The position in 1961 was quite simply that any chemical that increased the incidence of any form of cancer irrespective of dose or route of administration was by definition a carcinogen and therefore a potential risk for man. Not only was this the generally accepted view in the early 1960s but there were those who thought that tests involving parenteral injection should be used for food and other chemicals that were poorly absorbed from the gut (Boyland, 1950; Gross, 1961). Persuaded by this view, the medical establishment recommended the speedy withdrawal of iron dextran from the market despite its acknowledged value in the treatment of iron deficiency anaemia (British Medical Journal, 1960).

Golberg's response to this was to embark on a programme of laboratory research aimed at showing why the findings in rats were not applicable to humans. However, another quarter of a century had to elapse before the concept of non-genotoxic carcinogenesis began to trip off the tongues of cancer researchers.

Baker et al. (1961), using radiolabelled iron dextran, found that retention of iron at the injection site was higher in the rat — particularly the male rat — than in the mouse, rabbit, dog or man; and Golberg et al. (1961) reported that the local response to injected iron dextran — in the form of necrosis, accumulation of iron-laden macrophages, active fibroblastic activity and severe fibrosis — was more marked in the rat than in other species. By comparison histological sections prepared from gluteal injection sites in patients given courses of injections of iron dextran up to 3 years previously showed no necrosis or fibroblastic activity. In the light of these and other data, it was postulated that, by reducing the dose introduced into any one injection site, necrosis, fibrosis and the risk of neoplasia could be avoided. This proved not to be entirely true in the rat. Thus, when we (Roe et al., 1964) compared the response of rats given multiple injections of iron dextran into the same subcutaneous site with that in rats given the same number of injections but distributed between two, four or six different sites, we found that the total number of sarcomas that developed increased with the number of injection sites used, although the length of the latent interval between first injection and tumour development was inversely related to the number of injection sites.

It was also postulated that species in which iron was more efficiently removed from the injection site (e.g. rabbit and man) would be less susceptible than the rat to the induction of sarcomas. In practice, however, it proved easy to produce sarcomas in rabbits by giving them repeated intramuscular injections of iron dextran (Haddow et al., 1964).

Early on it was shown that the dextran moiety of iron dextran (Haddow and Horning, 1960) did not give rise to injection site tumours, and it seemed unlikely that iron per se was carcinogenic insofar as no increased incidence of cancer was ever seen at the sites (e.g. the liver) to which the metal was carried from the injection site (Golberg and
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both by injection and by feeding, but the injection test is
the more stringent and desirable". When he wrote this, he
foundations of our present-day understanding of non-
search to bear on the problem and began to lay the
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ciciency was the cause of pathologically low haemoglobin
stores are low (Davies, 1971) not all of the syringe-happy
those associated with the non-pregnant state. Further-
with levels of haemoglobin that are 10-15% lower than
normal pregnancy is associated with haemodilution and
injections which they did not need (Haddow, 1960). Some
larly pregnant women, were being given parenteral iron
injections led to the occurrence of a single case of injection-site
results of this investigation uninterpretable.

In my view the battle of giants ended as a draw. Whether the unnecessary use of parenteral iron prepara-
tions led to the occurrence of a single case of injection-site sarcoma in a human is very doubtful. However, it is
undoubtedly true that 30 years ago many people, particu-
larly pregnant women, were being given parenteral iron
injections which they did not need (Haddow, 1960). Some
of the physicians involved simply overlooked the fact that
normal pregnancy is associated with haemodilution and
with levels of haemoglobin that are 10–15% lower than
those associated with the non-pregnant state. Fur-
more, despite the fact that it has been shown that parenteral injection of iron is only of value if bone marrow iron
stores are low (Davies, 1971) not all of the syringe-happy
physicians took the trouble to establish that iron defi-
ciency was the cause of pathologically low haemoglobin
levels. The contribution of Golberg and his colleagues
was that they brought logic and careful laboratory re-
search to bear on the problem and began to lay the
foundations of our present-day understanding of non-
genotoxic mechanisms involved in carcinogenesis.

Cameo II: Does it get under your skin?

Boyland (1958) wrote "Food additives should be tested
both by injection and by feeding, but the injection test is
the more stringent and desirable". When he wrote this, he
was not out of tune with the thinking of the time. How-
ever, Leon Golberg was sure that this view was wrong,
and one of the first programmes of research that he
launched after his appointment as the first Director of
BIBRA in 1961 was to assess the value of subcutaneous
sarcoma induction as an index of carcinogenic activity.

During the years that followed there appeared a series of
papers by Paul Grasso, Leon Golberg, Sharat Gangolli
and Jean Hooson that steadily eroded faith in the appro-
priateness of the subcutaneous injection method for test-
ing substances for carcinogenicity, particularly substances
for which exposure was normally by the oral route.

Grasso and Golberg (1966b) reviewed many different
aspects of this problem. They distinguished between
‘true’ carcinogens, ‘weak’ carcinogens and substances
that act only non-specifically and indirectly to produce
carcinomas. ‘True’ carcinogens were further subdivided
into (i) those that produce sarcomas, and maybe other
tumours, at the site of injection plus tumours at sites
distant from the injection site, and (ii) those that only
produce tumours at distant sites. They considered the
influences of the dose administered, the solvents used and
the relationship between the production of sarcomas by
the injection of chemically unreactive compounds and
their production by implants of metal, glass, plastic and
other solid objects. They commented on the fact that
certain undoubted carcinogens had not been found to
produce sarcomas at the site of parenteral injection.
However, they were working before the importance of
metabolic activation of procarcinogens to carcinogens by
endogenous enzymes was understood. Their tentative
conclusion was that physicochemically-mediated trauma
leading to derangement of connective tissue repair can
lead non-specifically to the development of sarcomas. In
support of this tentative conclusion they were able to refer
to their findings in studies of changes at the site of
repeated injections of certain food colourings (Grasso and
Golberg, 1966a) and, of course, the results of studies on
iron dextran (vide supra). Gangolli et al. (1967) showed
that the nature and extent of local tissue reaction was not
closely related to retention of the injected material at the
injection site. More important determinants of local tissue
response were surface activity, lipid solubility and pro-
tein-binding activity, particularly if it led to alteration of
protein structure. Hooson and Grasso (1970) found that
etopic response to water-soluble true carcinogens was
quite different from that to those materials that produce
injection-site sarcomas. Instead of the marked and pro-
longed fibroblastic activity associated with the latter, there,
can occur an inhibition of fibroblastic proliferation and of
granulation tissue formation. Grasso et al. (1971) showed
how calcium ion concentration and modification of the
surface activity of the material injected could determine
both early local tissue response and risk of subsequent
sarcoma development.

In the light of this carefully conducted programme of
research, it would nowadays be difficult to find a respected
toxicologist who would recommend screening food con-
stituents, additives or contaminants for carcinogenicity
using parenteral injection as the route of administration.
Nevertheless, there are, unbelievably, still some who defend the use of intrapleural or intraperitoneal injection as a suitable method for evaluating possible carcinogenic risk from inhaled fibres and other particles (Pott et al., 1987). In the light of the research on subcutaneous injection carcinogenesis, it is my strongly held view that no reliance should be put on the results of injecting dusts or fibres into serosal spaces.

Cameo III: Beware of occlusive knickers

*Trichomonas vaginalis* is a venereal disease. Describing the situation in the 1950s, Duncan Catterall (1977) wrote:

“At the majority of clinics there were queues of sad-looking, unhappy women . . . with wet and stained underwear and poor morale. Nor was there any satisfactory treatment to offer them.”

Towards the end of 1954 Rhône-Poulenc began to look for a drug that would be effective against the causative parasite and thereby offer relief to the millions of women throughout the world who were infected with it. A step forward was made when Despois et al. (1956) found that a crude extract of the strain of *Streptomyces* was active against the parasite. The active principle was azomycin (Fig. 1). Numerous analogues were synthesized and tested and 4 years later Cosar and Julou (1959) reported the successful treatment of mice infected with *T. vaginalis* by the oral administration of metronidazole (Fig. 1). Soon after this, the drug was found to be effective clinically (Durel et al., 1959) and was christened ‘Flagyl’.

Since 1960, trichomoniasis has been a treatable disease. Local intravaginal use favoured initially, particularly in France, soon gave way to oral treatment, which proved more effective. Courses of treatment lasting over 7–10 days gave way to a single large-dose treatment (e.g. 2 g per person). The need to treat sexual consorts at the same time as patients themselves became widely recognized. Fortunately, drug resistance never became a problem, probably because there was no margin between the toxic concentration of metronidazole and the concentration that was lethal for the protozoon. Other nitroimidazoles were developed and marketed for the treatment of the disease but none proved more effective than metronidazole itself.

This might have been the end of the story had it not been for the chance observation of David Shinn, a dental surgeon at King’s College Dental School in London, and for the wisdom and dogged persistence of Dr J.A. McFadzean, the Research Director of the May & Baker subsidiary of Rhône-Poulenc. Shinn, who had for some time with only limited success been treating a woman for Vincent’s angina, a painful and distressing infectious disease of the oral cavity, was surprised when one day it suddenly cleared up. On enquiring, he found that the lady in question had also had problems at the other end of her body in the form of trichomoniasis. A course of Flagyl had been equally effective for her mouth and for her vagina. Shinn contacted McFadzean and a pilot study that confirmed the efficacy of metronidazole in the treatment of Vincent’s disease was reported (Shinn, 1962).

The question then was: why did Vincent’s angina respond to the drug? The disease had been reported to be associated with the presence in the mouth of large numbers of spirochaetes and fusiform bacilli, and although treatment with metronidazole led to the disappearance of these organisms from the mouth, another important development was necessary before the true cause of the disease and the reason for its response to metronidazole could be understood.

In the late 1940s the chances of a patient who had bowel surgery—even a simple appendicectomy—or a hysterectomy developing a post-operative wound infection ranged up to 50%. Swabs taken from many of the wounds were reported to be ‘sterile’. Nevertheless, wounds continued to produce pus and were very slow to heal.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of patients</th>
<th>Anaerobic sepsis rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole umbrella</td>
<td>74</td>
<td>1</td>
</tr>
<tr>
<td>No metronidazole</td>
<td>60</td>
<td>30</td>
</tr>
</tbody>
</table>

Study Group Report (1975)
The breakthrough in understanding this situation came with two discoveries. First a report appeared (Drasar et al., 1966) indicating that 98% of the bacteria present in the large intestine are anaerobic in type and belong to the Bacteroides group, whereas less than 0.1% are gram-negative bacilli (e.g. Escherichia coli). However, the recognition that many wound infections that produce apparently sterile pus are in reality due to obligate anaerobic bacteria had to await the development in the early 1970s of laboratory methods for growing these bacteria from swabs taken from patients. As soon as such methods became available, it became apparent that metronidazole was highly active in killing these bacteria (Nastro and Finegold, 1972).

In 1974 Trevor Willis, Director of the Public Health Laboratory in Luton and a leading authority on anaerobic bacteria, managed to persuade his clinical colleagues at the Luton and Dunstable Hospital to carry out a prophylactic trial of metronidazole as an ‘umbrella’ during hysterectomy (Study Group, 1974). As shown in Table 1, the results were dramatic (Report of Study Group 1975). Unfortunately, however, the medical establishment, which claims to be always on the look-out for breakthroughs, sometimes fails to recognize when they happen. The uphill struggle against scepticism that Willis and his colleagues faced is graphically described by McFadzean (1986), whose dogged determination to find a new use for an old drug met with comparable resistance within Rhône-Poulenc, and led to his being dubbed ‘Dr McFlagyl’.

Nowadays, as is well known, metronidazole is more or less routinely used as a prophylactic against post-operative infections by anaerobic bacteria in the course of bowel and gynaecological surgery. It also has a place in the treatment of numerous other infectious conditions now recognized as being due to obligate anaerobic organisms (Finegold, 1977; Willis, 1977). However, before this happy state of affairs emerged one further hurdle had to be cleared.

Long-term tests in rodents indicated that prolonged exposure to very high doses of metronidazole increased the risk of lung tumours and malignant lymphoma development in mice (Rust, 1977; Rustia and Shubik, 1972) and that heavy exposure of rats increased the incidence of a variety of neoplasms (Rustia and Shubik, 1979). Furthermore, positive results were reported in some tests for genotoxicity (Mittelman et al., 1976; Rosenkranz, 1977; Voogd et al., 1974).

The mutagenic activity of metronidazole is attributable to the nitroreductase activity possessed by the obligate anaerobic bacteria that it kills. Aerobic organisms and healthy mammalian cells do not possess such activity. The laboratory evidence of genotoxicity was, therefore, not relevant to man. A remarkable aspect of virtually all the long-term tests in rats, mice and hamsters was that exposure to metronidazole led to improved survival compared with controls. Overnutrition is associated with markedly increased incidences of lung tumours and malignant lymphoma in mice (Conybeare, 1980; Roe and Tucker, 1974). It is probable that, like other antimicrobial agents that affect the gut flora, metronidazole improves the nutritional status of mice and consequently has a similar effect to overfeeding (Roe, 1977). The failure of Rustia and Shubik (1979) to distinguish between fatal and incidental tumours and to age-standardize the data they obtained in their rat study rendered their findings uninterpretable.

Such data as have been published are consistent with there being no increased risk of cancer in humans after exposure to metronidazole. Beard et al. (1979) followed up 771 women who received metronidazole for trichomoniases during the period 1960 to 1969. A comparable group of 237 sufferers from the disease who received no metronidazole were available for comparison. In both groups the incidence of carcinoma of the cervix was higher than that for the general population. This was only to be expected in view of the well known association between the risk of this form of cancer and what is euphemistically referred to as ‘poor sexual hygiene’. Apart from this the only excess of cancer risk seen in the metronidazole treated group was four cases of lung cancer instead of only 0.6 cases expected. The four women in question were smokers. A follow-up under the Kaiser-Permanente programme of nearly 2500 women given metronidazole between July 1960 and August 1973 revealed no evidence of any excess of cancer at any site (Friedman and Ury, 1980).

Some time in the early 1980s I found myself attending a meeting of a US-FDA Committee concerned with the safety of using metronidazole in the treatment of trichomoniasis. To my amazement and horror a lady sitting next to me told those assembled that in her view there was no need to treat women with a proven carcinogen. She herself had suffered continuously from trichomoniasis for about 20 years but had found that she could easily live with it provided that she had a hot bath each day and avoided wearing occlusive knickers! I vividly remember the deafening noise of chairs being edged away from where she was sitting.

Happily today metronidazole is widely used both in the treatment of trichomoniasis and other protozoal diseases and in the prevention and treatment of numerous conditions caused by anaerobic bacteria, and the benefits in terms of reduced suffering and the average length of stay in hospital are enormous.

Cameo IV: Lumps and bumping off

Early in the 1970s I asked Richard Peto to help me to interpret the results of a long-term skin painting study in mice. Some of the treatments were associated with the development of malignant tumours of the skin and for humane reasons it was necessary to kill these animals. In consequence, there was an inverse relationship between survival and skin tumour incidence. But I wanted to know whether the different treatments to the skin were associated with different risks of development of lung tumours. Peto's first approach was to conduct an actuarial analysis...
which assumed that mice found at autopsy to have lung tumours died because of these tumours. I explained to him that most lung tumours in mice are benign and very slow growing. Thus, when a small lung tumour is found at autopsy in a mouse dying or killed because it has a skin tumour or for some other reason, there is no justification for regarding the lung tumour as in any way contributing to the animal’s death. Such an animal might well live for many months longer with its lung tumour growing slowly but not impairing its health and then die from some unrelated cause. Richard considered what I had said and questioned me about the likely contribution to death of various other internal tumours (such as hepatomas, interstitial cell tumours of the testis, C-cell tumours of the thyroid, benign phaeochromocytomas etc.) that are found in long-term rodent studies. I told him that in the vast majority of cases such lesions are simply incidental findings. After further consideration he concluded that this meant that the traditional way in which the results of carcinogenicity studies in animals had always been analysed had been entirely wrong.

Armed with this new insight and his own boundless energy Richard set about rectifying the matter, first with a short leading article in the British Journal of Cancer (Peto, 1974) and later, together with a quorum of other statisticians, with an unduly long Supplement to the IARC Monograph Series (Peto et al., 1980).

Nowadays, most experimentalists understand the need to try to distinguish between fatal and incidental lesions, and reports on carcinogenicity studies include lists of lesions considered to have contributed to premature death in laboratory animals.

However, the situation is still far from satisfactory. Some animals die for no apparent reason. Others are so

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**Table 2. Data relevant to carcinogenesis available from laboratory animals and man**

<table>
<thead>
<tr>
<th>Laboratory animals</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autopsy rate (%)</td>
<td>100</td>
</tr>
<tr>
<td>Thoroughness of autopsy</td>
<td>High</td>
</tr>
<tr>
<td>Reliability of data</td>
<td>Tumour incidence</td>
</tr>
<tr>
<td>Cancer mortality</td>
<td>Poor</td>
</tr>
<tr>
<td>Influence of therapy</td>
<td>High</td>
</tr>
</tbody>
</table>

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**Table 3. Percentage of deaths in 1991 for which autopsy was performed (WHO, 1992)**

<table>
<thead>
<tr>
<th>Country</th>
<th>All deaths</th>
<th>15–44</th>
<th>45–64</th>
<th>65+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hungary</td>
<td>49</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Sweden</td>
<td>37</td>
<td>76</td>
<td>56</td>
<td>36</td>
</tr>
<tr>
<td>England and Wales</td>
<td>26</td>
<td>62</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td>Scotland</td>
<td>15</td>
<td>57</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>USA</td>
<td>12</td>
<td>15</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>West Germany</td>
<td>8</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Netherlands</td>
<td>8</td>
<td>14</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Japan</td>
<td>4</td>
<td>8</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

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**Table 4. Examples of false negative and false positive rates (%) revealed by autopsy in the case of primary cancer of the lung**

<table>
<thead>
<tr>
<th>Country and Wales</th>
<th>Year</th>
<th>Correct</th>
<th>Wrong</th>
<th>Cancer only seen at autopsy</th>
<th>False -ve</th>
<th>False +ve</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>England and Wales</td>
<td>1966</td>
<td>227</td>
<td>111</td>
<td>190</td>
<td>45.6</td>
<td>32.8</td>
<td>(1)</td>
</tr>
<tr>
<td>Japan</td>
<td>1967</td>
<td>1488</td>
<td>305*</td>
<td>788</td>
<td>34.6</td>
<td>17.0*</td>
<td>(2)</td>
</tr>
<tr>
<td>Australia</td>
<td>1967</td>
<td>43</td>
<td>9</td>
<td>28</td>
<td>39.4</td>
<td>17.3</td>
<td>(3)</td>
</tr>
<tr>
<td>USA</td>
<td>1971</td>
<td>27</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>55.7</td>
<td>(4)</td>
</tr>
<tr>
<td>USA</td>
<td>1980</td>
<td>260</td>
<td>38</td>
<td>117</td>
<td>31.0</td>
<td>12.8</td>
<td>(5)</td>
</tr>
<tr>
<td>Scotland</td>
<td>1981</td>
<td>88</td>
<td>15</td>
<td>46</td>
<td>34.4</td>
<td>14.6</td>
<td>(6)</td>
</tr>
<tr>
<td>Italy</td>
<td>1986</td>
<td>72</td>
<td>27</td>
<td>62</td>
<td>46.3</td>
<td>27.3</td>
<td>(7)</td>
</tr>
</tbody>
</table>

*Total includes only cases diagnosed as cancer of other sites.

**References:** (1) Heasman and Lipworth (1966); (2) Takeda and Kobayashi (1967); (3) Willis (1967); (4) Rosenblatt et al. (1971); (5) Cechner et al. (1980); (6) Cameron and McGoogan (1981 a,b); (7) Mollo et al. (1986).
full of potentially ‘fatal’ lesions that it is difficult to know how they clung to life for so long and appeared so well.

Faced with such difficulties, it is all too common practice for computers to make the decisions about which lesions contributed to death. Thus, the computer may be programmed to list any malignant neoplasm, irrespective of its site or size, as such a factor. Where this happens, small well differentiated doubly malignant lesions that happen to lie in the path of the microtome knife are bracketed together with massive lesions described in graphic terms by prosecutors.

I doubt whether it will ever be possible to produce accurate cancer mortality data for laboratory rodents. However, there is certainly scope for improving the way in which things are done at present.

In any case, there is, and will remain, a serious gap between the kind of data produced from carcinogenicity studies in rodents and the kind of data available to epidemiologists concerned with cancer incidence in man (see Table 2). For most kinds of cancer arising internally the only data that are available for humans are mortality data. Currently, very low autopsy rates (Table 3) (e.g. only 3% in the USA and only 3% in Japan for persons over 65), combined with serious discrepancies between clinical and autopsy diagnoses (e.g. Abrahamson et al., 1971; Alderson et al., 1983; Heasman and Lipworth, 1966; Lee, 1993; Rosenblatt et al., 1971; Royal Colleges Report, 1991; Takeda and Kobayashi, 1967; Willis, 1967) and also discrepancies between findings at autopsy and entries on death certificates (James et al., 1955; see Lee, 1993 for review; Steer and Land, 1976) render even these data extremely inaccurate. As an illustration of how unreliable human cancer mortality data are, the results of seven studies on lung cancer are summarized in Table 4.

Thus, the position is that for small non-fatal neoplasms arising in internal organs there is no reliable source of data for humans. By contrast, for laboratory animals we have an abundance of data derived from detailed and more or less standardized autopsy procedures on the incidence of benign and malignant neoplasms but, for the reasons discussed above, only rather unreliable cancer mortality data.

On the accuracy of the post-mortem diagnosis Wilson (1966) wrote “... in justice and honesty it must be admitted that in a certain number of patients—about 20%—more or less mystery and confusion still prevailed after post-mortem examination”. Despite my claim to be a pathologist, I accept that the autopsy room is not the ‘palace of truth’ it has been claimed to be by some pathologists and feared to be by some clinicians. It would be less misleading to think of it as a place where one can come closest to the truth about the cause of death.

In conclusion, it seems to me a serious matter that the importance of the distinction between tumour incidence data derived from animal tests and human cancer mortality data is largely unknown to, and unappreciated by, activists, medical science writers, and many of those who sit on regulatory bodies and who advise politicians.

Cameo V: Danger in the air: beware of consensus

I was born and reared in the sulfurous fog of south-east London long before the Clean Air Act. Staying indoors provided me with little protection from air pollutants because only one room in our house was heated. In this small living room my father continuously smoked his pipe, the open fire intermittently delivered its fumes into the room instead of up the chimney, and my grandmother, having unilaterally declared south-east London to be a moth-free zone, sat in a haze of camphorous fumes. I look back in wonder that I both tolerated and survived all these insults to my lungs. By contrast, I am today thoroughly intolerant of intrusive odours and particularly of irritation from other people’s tobacco smoke.

In the early 1950s, laboratory studies in the field of tobacco carcinogenesis were confined to applying tobacco smoke condensate repeatedly to the dorsal skin of mice. In the early days negative results were often obtained either because of the Scylla of insufficient dosage, or because of the Charybdis of excessive dosage, which led to nicotine poisoning. Even if these hazards were successfully circumnavigated there remained a high risk of premature death from intercurrent disease. Eventual success in producing skin tumours with whole smoke condensates (Wynder et al., 1953) led on to skin painting experiments using fractions and sub-fractions of the condensate in the hope of pinpointing the carcinogen or carcinogens responsible for both the skin tumours in mice and the lung cancers in smokers. A sub-fraction of the neutral fraction that contained, inter alia, the polycyclic aromatic hydrocarbons (PAH) and related compounds proved to be most active (Whitehead, 1977). However, irritants in other fractions of the condensate, such as the phenolic fraction, had previously been shown to exhibit a tumour-enhancing effect (Roe et al., 1959). In the case of the PAH in the neutral fraction, attention was focused on the known carcinogen, 3,4-benzopyrene (BP), because its characteristic UV absorption spectrum rendered it easier to measure than other constituents of this fraction. However, we soon showed that this particular carcinogen contributes little to the carcinogenicity of smoke condensate for mouse skin (Roe, 1962). The tobacco industry spent huge amounts of money on such research, hoping, of course, that a single culprit carcinogen would be identified and that by a bit of clever manipulation with the raw tobacco or with the smoking process non-carcinogenic cigarettes could be produced.

Unfortunately, no single constituent of smoke condensate that could explain all its carcinogenic activity for mouse skin was found. Consequently, attention was switched to reducing the amount of tar delivered by cigarettes and to developing tobacco substitutes that, on pyrolysis, produced a simpler smoke with a lower PAH content. The first generation of products containing tobacco substitutes, which, incidentally, were given no tax advantage by Government, failed to satisfy smokers. Because of this and because of vigorous and, in my opinion, misguided opposition by anti-smoking cam-
There were two possible approaches to exposing laboratory animals to tobacco smoke by the inhalation route. One was to confine them within a chamber into which fresh smoke was introduced, either continuously or intermittently. The other was to enclose each animal separately within a tube with its nose protruding at one end and to fix that end of the tube to a cylinder through which fresh smoke was passed continuously or intermittently. The latter method had the advantages of avoiding the absorption of smoke constituents through the intact skin and of ensuring that the smoke to which animals were exposed was fresh, but it had the great disadvantage of being extremely labour intensive.

With both types of approach nicotine poisoning and carbon monoxide poisoning were both potentially serious problems. The human smoker can interrupt exposure whenever he/she feels a bit queasy but a rat in a chamber or tube cannot do so. The only solution was to cut down the rate of dosing and/or to arrange for smoke exposure to be intermittent. A further insuperable problem was that by either method of exposure, all the smoke taken into the lungs of animals passed first through the fairly efficient filtration system provided by the nose, whereas most of the smoke that humans take into their lungs enters through the mouth. It was seemingly impossible to expose the lungs of rats and mice to as much smoke as a dedicated smoker can get into his/her own lungs. In one rat experiment (Davis et al., 1975) the most prominent effect of exposure to smoke was a reduction in the incidence of mammary tumours (Table 5). Such effects on the lungs that were seen—including increased incidences of pigment-laden macrophages, cuboidal/columnar metaplasia of alveolar epithelium and squamous metaplasia of alveolar epithelium—were of doubtful relevance to the induction of lung cancers of the types associated with smoking in humans.

Looking back now, it is my view that laboratory research involving the exposure of animals to smoke by way of the inhalation route provided very few useful insights into the mysteries of tobacco carcinogenesis. However, some benefit certainly did stem from these experiments. New methods for exposing animals to chemicals by way of the inhalation route, for inhalation dosimetry and for the pathological assessment of the effects of exposure to industrial chemicals, fumes and dusts were developed—very often by those who had cut their teeth on tobacco smoke inhalation studies. For such chemicals, standard techniques are now available and widely used.

### Table 5. Effect of inhalation exposure of female rats to cigarette smoke (10 cigs/wk)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>0</th>
<th>80</th>
<th>120</th>
<th>Total lung tumours</th>
<th>Total mammary tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoke</td>
<td>408</td>
<td>143</td>
<td>61</td>
<td>4</td>
<td>37</td>
</tr>
<tr>
<td>Sham</td>
<td>102</td>
<td>91</td>
<td>35</td>
<td>0 NS</td>
<td>40**</td>
</tr>
</tbody>
</table>

**NS = not significant  ** = $P < 0.01$

from Davis et al. (1975).
Their relevance to environmental air pollution problems, on the other hand, is more dubious. The standard experimental protocol involves exposing animals to a test material for 6 hours per day on 5 days per week, excluding public holidays. Exposure to outdoor and indoor air pollutants, however, may extend through 24 hours per day on 7 days per week—as sometimes did my exposure to London smog and camphor when I was a child. For many obvious reasons, one needs to be very careful in extrapolating from short-term high level exposure to long-term low level exposure.

Despite all the research that has been carried out, the overall position with regard to tobacco smoke is that we do not know which, if any, of the animal carcinogens that have been identified in smoke, is important. It is not certain that the PAH in the particulate phase are really important. Nor, despite much research of high quality (Hoffmann, 1989) is the role of tobacco-specific nitrosamines really clear.

25 years ago we (Roe and Kearns, 1967) reported that an acetone extract of particulate air pollutants collected by a sampler on the roof of St Bartholomew's Hospital was some 15–20 times more carcinogenic, weight for weight, than cigarette smoke condensate (Table 6). Three times weekly application of 0.25 ml of the acetone extractable material from 2 mg of particulate material collected on a Cambridge filter readily induced benign and malignant skin tumours in mice.

There are many members of the medical profession and of the general public who are so concerned with the strong association between cigarette smoking and lung cancer, that they regard it as justifiable to use any means to stop people from smoking. Among the means presently used is to make statements about the numbers of lung cancer deaths attributable to exposure to environmental tobacco smoke. From a logical viewpoint such calculations are unconvincing, if not ludicrous. Epidemiology is a blunt tool (Bailar, 1989) and one that is clearly too blunt to measure a possible small difference in lung cancer between non-smokers married to smokers as compared with non-smokers married to non-smokers. In such groups, one cannot titrate risk against actual measurements of exposure to environmental tobacco smoke over a lifetime since no data for exposure exist, and in none of the 29 analysable studies of this kind (see Lee, 1992) was it possible to control reliably for all the other factors known to be important as determinants of lung cancer risk, such as radon (Clarke and Southwood, 1989), bird-keeping (Holst et al., 1988; Kohliemeier et al., 1992), outdoor air pollution (Roe and Walters, 1965), indoor air pollution (Chen et al., 1990), genetic factors, occupational factors and diet (Koo et al., 1988). People’s statements about their own present and past smoking habits are extremely unreliable (Lee, 1988). And furthermore, mortality data for lung cancer are grossly unreliable (Table 4).

All we know for certain is that environmental tobacco smoke can be irritant to the nose and eyes and aggravate the symptoms of asthma. Whether these short-term effects have more serious long-term consequences has not been established. Unfortunately, the toxicologist has not been able, so far, either to support or refute the claims and counter-claims made in relation to lung cancer risk from environmental tobacco smoke, and decisions concerning the existence and extent of hazards are being made by what is billed as consensus (e.g. EPA, 1992). Some of the biggest mistakes in history have been based on consensus thinking. As pointed out by Skrabanek (1990), consensus should never be regarded as a substitute for scientifically based facts.

Cameo VI: On the road to Damascus

"And as he journeyed, he came near Damascus: and suddenly there shined round about him a light from heaven" (Acts, 9 verse 3)

The development by Bruce Ames (Ames, 1971; Ames et al., 1973) of a quick, cheap and sensitive test system for genotoxicity based on Salmonella typhimurium, had an electrifying effect on cancer research relevant to the detection of environmental carcinogens. First came the creed and then unrelenting effort to exploit it. The first version of the creed was “All carcinogens are mutagens”. But this was quickly replaced in many people’s minds by “All mutagens are carcinogens”. The discovery that inert chemicals can, by what was called metabolic activation, be converted into mutagens provided a spur to unremitting efforts to show that any chemical that predisposes to cancer is in fact a mutagen or can be converted into a mutagen. Undeterred by negative results in 20 or more tests for genotoxicity, the ardent believer in the new faith would simply press on developing new tests for genotoxicity, being certain in his/her own mind that sooner or later the rogue chemical would be shown to be a mutagen.

The faith and the creed led to all sorts of useful substances coming under suspicion of carcinogenicity. It also led to the emergence of the 'one-molecule men', that
is to say of a clique of individuals who were prepared to argue that the exposure of the DNA of one body cell to one molecule of a mutagen could be enough to cause a cancer to arise.

As a pathologist who was seeing cancer in high incidence in untreated control animals as well as in animals deliberately exposed to potent genotoxic carcinogens, and as an experimentalist who was observing increased incidences of liver tumours in mice fed on diets containing increased levels of ordinary food ingredients such as groundnut oil (Gellatly, 1975) or sucrose (Hunter et al., 1978) I was never tempted to chant these creeds. And later when I became aware of the increased incidences of adrenal medullary tumours and testicular (interstitial-cell) tumours in rats fed on a high lactose diet (Sinkledam et al., 1983; Roe and Baer, 1985), I became convinced that genotoxicity was not a sine qua non of carcinogenicity. However, for a long time I had no insight into any alternative explanation.

The induction of malignant cancers by natural hormones—for example the induction of pituitary and ovarian cancers by removing the ovaries from an animal from their natural site and then implanting one of them back into the spleen (Biskind and Biskind, 1944)—was a clear example that naturally occurring substances as distinct from xenobiotic chemicals were capable of causing cancers to develop.

It was only when I read the paper by Bruce Ames (1983) that the picture began to become clear. His sudden recognition that all sorts of naturally occurring plant and other materials were genotoxic was seemingly a St Paul-on-the-road-to-Damascus-like revelation. But it was the other contents of that paper and of the later paper by Ames and Saul (1987)—particularly the fact that genotoxins are formed endogenously during the conversion of foodstuffs to energy—that really opened my eyes to the light. At long last I could see possible explanations of how natural hormones might cause cancer and of how dietary restriction protected against it. From that time on I could see how non-genotoxic substances might indirectly give rise to genotoxic damage and to the cell-structure damage associated with ageing. ‘Non-genotoxic carcinogenesis’ became an acceptable concept and what I believe to be was the first international conference on ‘Nongenotoxic Mechanisms in Carcinogenesis’ was held in Cold Spring Harbor in 1987 (Cold Spring Harbor Laboratory, 1987).

Cameo VII: Think before you eat

During the 1960s whilst I was at the Chester Beatty Research Institute I ran head-on into the problem of high tumour incidence in untreated control groups of animals. In mice the commonest, so-called ‘spontaneous’ tumours were of the lung, liver and lymphoreticular system. In rats, mammary, pituitary and other endocrine tissues were the most commonly affected. Unfortunately, the sites most frequently involved in spontaneous tumour development were exactly the same as those that constituted the endpoints in carcinogenicity studies. Irrespective of statistical significance, I was simply not persuaded that enhancement of tumour incidence from say 30% to 60% by a certain age could be taken as reliable evidence of carcinogenicity. Genetic factors were clearly implicated insofar as there were big differences between strains in spontaneous tumour incidence at particular sites. However, no strain of either rats or mice was immune to the problem in studies of 2 years’ duration or longer. This situation, combined with the fact that the induction of sarcomas at the site of subcutaneous injection or implantation of virtually any chemical material into rodents was being accepted as proof of carcinogenicity was in my view making something of a nonsense of carcinogenicity testing.

My despair was only relieved when early in the 1970s I heard Mary Tucker present her data about the dramatic

<table>
<thead>
<tr>
<th>Table 7. Some effects of restricting calorie intake of 80% of ad lib. in 1200-rat Biosure study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature body weight</td>
</tr>
<tr>
<td>Survival to age of 133 wk</td>
</tr>
<tr>
<td>Reduction in non-neoplastic ageing-associated disease*</td>
</tr>
<tr>
<td>Polyarteritis</td>
</tr>
<tr>
<td>Chronic myocarditis</td>
</tr>
<tr>
<td>Prostatitis</td>
</tr>
<tr>
<td>Reduction in neoplastic disease (benign and/or malignant)*</td>
</tr>
<tr>
<td>Any site</td>
</tr>
<tr>
<td>Epidermis and/or adnexa</td>
</tr>
<tr>
<td>Subcutaneous</td>
</tr>
<tr>
<td>Mammary</td>
</tr>
<tr>
<td>Pituitary – anterior lobe</td>
</tr>
<tr>
<td>Pituitary – intermediate lobe</td>
</tr>
<tr>
<td>Pancreas – islet-cell</td>
</tr>
<tr>
<td>Pancreas – exocrine</td>
</tr>
<tr>
<td>Lung</td>
</tr>
</tbody>
</table>

* = Age-standardized incidence: (•) = P < 0.1; • = P < 0.05; •• = P < 0.01; ••• = P < 0.001.
Table 8. Effect of ad lib. feeding compared with restriction to 80% of ad lib. on relative risk of development of fatal or potentially fatal malignant neoplasms

<table>
<thead>
<tr>
<th>Relative risk (95% confidence limits)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Of premature death</td>
<td>2.40 (1.61–3.59)</td>
<td>3.60 (2.42–5.59)</td>
</tr>
<tr>
<td>Of developing fatal or potentially malignant neoplasms</td>
<td>4.80 (2.73–8.74)</td>
<td>3.34 (1.97–5.66)</td>
</tr>
</tbody>
</table>

(see Roe and Lee, 1991)

Table 9. Relative risks (RR) of (a) dying prematurely and (b) developing one or more benign or malignant neoplasms before the age of 133 wk in relation to body weight at the age of 29 wk

<table>
<thead>
<tr>
<th>Body weight quintiles at 29 wk</th>
<th>Sex</th>
<th>Very low</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>Very high</th>
<th>Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>118</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>120</td>
<td>120</td>
<td>118</td>
<td>122</td>
<td>116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M or F</td>
<td>1.00</td>
<td>1.23</td>
<td>1.56</td>
<td>1.85</td>
<td>2.56</td>
<td>P &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1.00</td>
<td>1.49</td>
<td>1.36</td>
<td>1.97</td>
<td>2.11</td>
<td>P &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>1.00</td>
<td>1.69</td>
<td>2.43</td>
<td>2.78</td>
<td>3.22</td>
<td>P &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1.00</td>
<td>1.39</td>
<td>1.33</td>
<td>1.96</td>
<td>1.69</td>
<td>P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>M &amp; F</td>
<td>1.00</td>
<td>1.36</td>
<td>1.76</td>
<td>2.71</td>
<td>2.93</td>
<td>P &lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

M = male  F = female

I believe that, at long last, heed is beginning to be taken of the situation, but there are still many who find reasons for not wanting to face the facts. Among the reasons is a belief that tumours can only arise because there has been exposure to a xenobiotic carcinogenic agent and that high spontaneous tumour incidence is merely a marker of high susceptibility to xenobiotic carcinogens. However, many naturally occurring chemicals are genotoxic and potentially carcinogenic and cellular DNA is constantly being damaged by electrophiles generated during the normal metabolism of foodstuffs, particularly fats. Next, there are those who think that the caloric restriction of laboratory rodents is unnatural and/or unkind. It is of course, nothing of the sort. Caged, bored animals, like frustrated humans, overeat for the want of anything more interesting to do. Those who run zoos or dog kennels know this very well. And those who have observed the effects of slight dietary restriction in rats and mice know that the animals are not stunted in terms of bone length or brain reductions in spontaneous tumour incidence that could be brought about by simple dietary restriction. I persuaded her to join me in a joint presentation about the deficiencies in toxicity testing (Roe and Tucker, 1974) and ever since then have regarded it as a mission in life to convey the essential message that the ad lib. feeding of laboratory rodents with unnecessarily highly nutritious diets constitutes overfeeding, which predisposes to premature ageing and death, and to the development of a wide variety of benign and malignant neoplasms. The literature describing all this is abundant, going back to 1935 (Berg and Simms, 1960; Conybeare, 1980 and 1988; Deerberg et al., 1990; McCay et al., 1935; Masoro, 1984; Masoro et al., 1989; Nolen, 1972; Ross and Bras, 1965; Ross et al., 1983; Simms and Berg, 1962; Tannenbaum, 1940 and 1942; Tucker, 1979; Weindruch and Walford, 1988; Yu et al., 1982) but I feel no shame in adding to it further (Roe, 1981 and 1991; Roe and Lee, 1991, Roe et al., 1991; Salmon et al., 1990; Turnbull et al., 1985).
size but are slimmer, brighter, more generally active and have healthier skins and hair than animals fed ad lib. Thirdly, there are those who have over many years collected data from studies in which animals have been fed ad lib. and realize that these collected data would be of little value if they switched to a calorie-restricted feeding regimen. The need to create and maintain databanks does not justify the perpetuation of bad science, and there can be no doubt that carcinogenicity tests conducted under conditions in which the results are hopelessly confused by the consequences of overfeeding constitute very bad science. Elderly overfed rats in very considerable endocrine disarray are most certainly not models suitable for detecting carcinogenic risk for humans. Untreated female rats of strains commonly used for routine carcinogenicity tests frequently exhibit close to 100% incidences of pituitary and mammary tumours. There is no sub-group of the human population that can match this. Slight diet restriction goes a long way to normalizing this situation. Thus, it is my view that, if long-term animal tests for carcinogenicity have a future, it must be one that involves animals that, if untreated, remain in normal physiological and hormonal status during the study period.

In the 1200-Rat Biosure Study (Roe, 1991) the full results of which are still being prepared for publication, we confirmed the findings reported previously in many other studies with regard to the effects of restricting calorie intake to 80% of ad lib. Some of the main findings are summarized in Table 7.

In thinking about the interpretation of these findings in relation to man, we tried to take into account the fact that there are no reliable data for the incidence of benign neoplasms in humans because of low autopsy rates. The only data available to epidemiologists—and these are seriously inaccurate (Heasman and Lipworth, 1966; Slavin et al., 1991)—are cancer mortality data. Accordingly, we endeavoured to calculate cancer mortality rates for the rats. This involved assuming that malignant tumours found in animals that were killed for humane reasons caused the sickness for which they were killed. The effects of slight dietary restriction on cancer mortality were marked and highly statistically significant in both sexes (Roe and Lee, 1991) (Table 8). Moreover, the percentages of our calorie-restricted rats that developed potentially fatal malignant cancers (i.e. 38.7% of males and 35.5% of females) were not dissimilar from those in humans. At this point I should emphasize that these figures are for rats not deliberately exposed to any carcinogen—but simply fed on a standard laboratory diet.

The study as a whole involved several different diets and dietary regimens. When ignoring these differences, we found that the risks of premature death and of developing a malignant neoplasm were highly correlated with body weight 6 months after the start of the study (Table 9). Others have reported similar findings (e.g. Rao et al., 1987).

We live in an age in which we are being bombarded by advice on what to eat and what not to eat. Claims and counter-claims as to what is riskier and what is safer are not infrequently based on laboratory experiments involving the feeding of qualitatively different diets. It needs to be said loudly and clearly that the results of research on which such claims are made are wholly unreliable unless conducted under isocaloric conditions. It is the total calorie intake and not dietary composition that is the main determinant of health risks from food in laboratory animals (Masoro, 1988; Weindruch and Walford, 1988). Particularly pertinent in this regard are numerous investigations of the influence of diet on the development of mammary cancer in female rats previously exposed to a known potent carcinogen. Even if one can accept that rats given a large dose of a xenobiotic carcinogen (7,12-dimethylbenzanthracene) on about the 60th day of life are suitable models for studying breast cancer development in women, it makes a nonsense then to compare the effects of qualitatively different diets without controlling for calorie intake when one knows that calorie intake is a major determinant of whether and when mammary cancers will develop. A quotation from Ip (1991) makes the point quite clearly in relation to the DMBA-induced mammary tumour model: “Calorie restriction, even in the presence of a high fat intake, is more striking than a decrease in dietary fat in suppressing the development of induced mammary cancer”.

The most obvious explanation of the relationships between calorie intake, premature ageing and cancer is that oxidants that damage both cell protein and DNA are generated during the metabolism of ordinary foodstuffs, particularly fats, and although efficient repair systems exist there is a slow build-up of unrepaired damage with the passage of time (Ames, 1989 and 1990; Ames and Saul, 1987). There is a brief period in the cell cycle when the two strands of DNA are separated. During this brief period, DNA repair is hampered and there is an increased risk of damage being fixed and passed on to subsequent generations of cells. Cell-turnover rates are higher in overfed, that is ad lib. fed, rats and mice (Lok et al., 1990) (Table 10) and consequently unrepaired DNA damage

<table>
<thead>
<tr>
<th>% Inhibition of $[3H]$thymidine labelling index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary gland</td>
</tr>
<tr>
<td>Bladder epithelium</td>
</tr>
<tr>
<td>Dermis</td>
</tr>
<tr>
<td>Oesophageal epithelium</td>
</tr>
<tr>
<td>Colon-crypt cells</td>
</tr>
</tbody>
</table>

++ = $P < 0.05$; + = $P < 0.01.$

(from Lok et al., 1990)
and ageing-related damage to the structural components of cells accumulate more rapidly in them. During the last 2 years or so these concepts have been hotly debated, particularly in the columns of the journal Science (Abelson, 1990; Ames and Gold, 1990; Cohen and Ellwein, 1990; Weinstein, 1991), and the arguments are not over yet. Weinstein (1991) may be right in pointing out that the spectrum of DNA damage produced by oxidants released during the metabolism of food might well be different from that produced by xenobiotic genotoxic carcinogens. He may be right, too, in concluding that mitogenesis is only one factor in carcinogenesis. We still have to face the difficult problem of distinguishing the potentially more important damage to the DNA and proteins of stem cells and damage to non-stem cells. The temptation to conclude that carcinogenesis and ageing have the same cause needs to be tempered by the fact that most of the pathological changes associated with ageing diseases involve non-stem cells, which may or may not have abnormal DNA, whereas the whole edifice of modern day cancer research is built on the belief that genomic abnormality is invariably present in cancer cells.

Clearly, then, there is still a long way to go before we understand the relationship between calorie intake, premature ageing and cancer. Nevertheless, our present lack of understanding provides no excuse for ignoring data that point to calorie intake being a major determinant of the risk of developing all forms of cancer. Epidemiologists and toxicologists who fail to control for calorie intake in their studies are in danger of reaching false conclusions.

General conclusions

Although I have talked diversely on seven different topics, there is a theme that has run through them all. It is that damage to DNA by xenobiotic genotoxins is not the only determinant and perhaps not even the most important determinant, of whether and when a human or a rat develops cancer. There is at present no reason to suspect that mutations, usually multiple and successive, do not occur at some stage in cancer development, but they may well occur as a consequence of internally generated genotoxins during the metabolism of foodstuffs or in association with inflammatory processes. I have no doubt that necrosis followed by regenerative hyperplasia, particularly if this is a repeated sequence, increases the risk of cancer development. Against this background it would have been a great mistake to have banned the use of metronidazole because of its effects on bacteria in vitro or because in vast dosage it increased the incidence of certain commonly occurring tumours in overfed rodents.

Attention is drawn first to the Gulf between cancer mortality data as used by epidemiologists and tumour incidence data as used by toxicologists, and secondly to the serious inaccuracy of human cancer mortality data. Partly for the latter reason, and more importantly because of poor exposure data and the difficulty of controlling for multiple confounding variables, epidemiologists can neither establish nor exclude low levels of cancer risk from exposure to factors such as environmental tobacco smoke. However, there is no excuse for experimentalists not to control for known confounders, such as calorie intake, in the course of tests for carcinogenicity in laboratory rodents.

Acknowledgements

I am grateful to many people for their help and advice during the preparation of this manuscript. My particular thanks are due to Liza Roe for typing and co-ordinating numerous versions and revisions of the text, to Alan Dagnall and Malcolm Wright for preparing the transparencies used in the lecture on which the text is based, and to Paul Grasso, Peter Lee, Jeffrey Cohen, Trevor Willis and Jim McFadzean for help with specific parts of the text.

References

Cameo I


**Cameo II**


**Cameo III**


**Cameo IV**


Cameo V


Cameo VI

Mycotoxins in the Diet

The Steering Group on Chemical Aspects of Food Surveillance has published its third report on mycotoxins, which it last reviewed in 1987 (BIBRA Bull. 1987, 26, 117).

Patulin in apple juice, which has been the subject of much press comment, was found at higher levels than previously. This was probably due to the growing popularity of fresh, cloudy juices, often produced from fruit held in long-term storage. Some 17 samples of cloudy juices contained concentrations in the range to 434 mg/kg, with a median value of 28 mg/kg. In 15 clear juice samples concentrations ranged only up to 118 mg/kg, with a median value below 10 mg/kg (the detection limit), and in all but one sample were below 50 mg/kg. Apple juice manufacturers have now agreed to adhere to GMP by avoiding the use of mouldy fruit, and a follow-up survey is planned.

The proportion of whole dried figs exceeding the new statutory limit for aflatoxins of 4 mg/kg (ibid 1993, 31, 55) fell from 26% to 16% between December 1988 and April 1992, although a high maximum level of 427 mg/kg was found during the last sampling year. The proportion of fig pastes exceeding the limit likewise declined from 50% to 14% over the same period, and in this case the maximum level also fell, from 165 to 15 mg/kg. Some 28% of pistachio nuts sampled during 1991–92 contained more than 4 mg/kg, and 25% exceeded the 10 mg/kg limit for products that are to be further processed, the maximum detected being 813 mg/kg. All consignments found to contain more than 10 mg/kg were refused entry by port authorities, and none of 109 retail samples from the Norwich area proved to contain more than 4 mg/kg. Discussions with fig and nut importers and the Turkish and Iranian producers have now taken place to improve control measures, including screening and analysis before shipment.

‘Crunchy’ peanut butter continued to contain more aflatoxin than ‘smooth’ varieties, and the proportion of both types containing more than 4 mg/kg rose between 1986 and 1991, from 11% to 31% for crunchy and from 10% to 19% for smooth varieties, although maximum concentrations remained similar (53 mg/kg in 1986 and 51 mg/kg in 1991). It was concluded that industry will need to change its sorting procedures to ensure consistent compliance with the new regulations. Aflatoxin B1 levels in animal feedingstuffs consistently complied with the applicable regulations (ibid 1988, 27, 344), and aflatoxin M1, levels in milk and cheese presented no cause for concern.

Ochratoxin A concentrations ranged up to 45 mg/kg in cereals, 9.3 mg/kg in pig kidney and 1.8 mg/kg in black pudding, although only 10–29% of different products sampled contained more than the detectable limit of 1 mg/kg. Assuming that each foodstuff contained the maximum level found for that sample type, it was estimated that extreme consumers might ingest 9.5 ng/kg body weight/day. This is below the JECFA provisional tolerable weekly intake of 112 ng/kg body weight (ibid 1990, 29, 293; ibid 1991, 30, 150 & 334), which, however, was based on the no-effect level for kidney damage and did not take account of subsequent evidence of genotoxicity. Ochratoxin A has recently been reported in Swedish human blood plasma samples, and a survey of UK blood is now planned.

Moniliformin was found in all but three of 36 maize products (whole maize, meal and flour) sampled in 1990, at levels in the range 50 to 250 mg/kg. As certain ethnic groups consume unprocessed maize as a dietary staple, periodic surveillance will continue, although its priority will be low.

In comments on the report the CoT welcomes the new aflatoxin regulations, and recommends that surveillance of affected products should continue. As ochratoxin A has mutagenic, carcinogenic, teratogenic and immunotoxic effects and is persistent within the body, contamination should be reduced to the lowest level technologically achievable. Moniliformin produces myocardial degeneration in rats, for which a no-effect level has not been established, and is suspected to contribute to Keshan disease (cardiomyopathy) in China. As few of the UK population are likely to consume unprocessed maize products regularly, the levels found are not considered to be of particular concern, but the effects of processing should be investigated, since processed maize products are common items in the UK diet. Patulin is mutagenic and has adverse reproductive, immunological, neurological and gastro-intestinal effects in rodents, and levels should therefore be reduced as much as possible. Surveillance should be extended to other food products containing...
apples, and continued to ensure that effective control measures are introduced by industry. In future surveys it should be ascertained whether there are other important fungal metabolites in the UK diet, the toxicity and incidence of which require further investigation.

This advice is endorsed by the FAC, who also recommend that the apple juice industry should introduce quality control measures to keep patulin below an action level of 50 mg/kg. A code of practice for apple juice production should be drawn up, advisory material on the dangers of patulin should be prepared, targeting in particular the smaller producers of apple juice, and the public should be warned not to use mouldy fruit in home juice production.


ABSTRACTS FROM THE LITERATURE

Cola drinks
A time-related increased incidence of DNA damage (adducts) was found in the liver of mice given cola drinks in place of drinking water for 8 wk. Myristicin, a naturally occurring compound found in several foods including nutmeg and mace, was identified as the major component responsible. DNA adducts were also detected in foetal liver when pregnant mice were intubated with myristicin (Randerath et al., Biochemical and Biophysical Research Communications 1993, 192, 61).

Caffeine
The effect of exposing pregnant rats to caffeine on the reproductive parameters of their offspring and outcome through eight subsequent litters has been studied by Australian investigators. Female rats (F₀) received either 30 or 60 mg caffeine/kg body weight daily by stomach tube from day 2 to 21 of pregnancy. Female offspring (F₁) of these animals were then bred through eight consecutive matings. The F₂ offspring, most notably in the first litter derived from mothers given the top dose of caffeine bled heavily when giving birth. Female offspring (F₁) of these animals were then bred through eight consecutive matings. The F₂ rats derived from mothers given the top dose of caffeine bled heavily when giving birth to the F₃ generation, and there was a dose-related increase in the number of stillborn litters. Birth weight was often increased in the F₂ offspring, most notably in the first litter (Pollard and Claassens, Reproductive Toxicology 1992, 6, 541).

Formaldehyde resins
US investigators examined all records for patients at two dermatitis clinics between January 1988 and April 1990 (a total of 1022 patients) to determine the prevalence of positive patch-test reactions to formaldehyde-based textile resins (used in 'permanent press clothing') and the clinical and demographic patterns associated with textile resin allergy. 17 patients with allergy to formaldehyde textile resins were identified. Interestingly, in five of these cases, allergy to formaldehyde itself could not be demonstrated (Fowler, Jr et al., Journal of the American Academy of Dermatology 1992, 27, 962).

Styrene
Performance in a number of psychological tests was poorer in 30 French dockyard workers with daily exposure (for a mean period of 5 yr) to low levels of styrene than in 30 unexposed carefully matched control workers. Subjects were tested on Monday morning and again in the evening following a daily exposure to an average 23 ppm (range 4 to 55 ppm). Exposed workers performed less well than controls both in the morning and evening tests. However, there was no indication of a deterioration in performance within the group following the day's exposure, nor did those with 8-14 yr of exposure perform significantly worse than those with only 1 yr of exposure. The current UK maximum exposure limit for styrene is 100 ppm (8-hr TWA) (Jégaden et al., International Archives of Occupational and Environmental Health 1993, 64, 527).

Dinitrotoluene
An increased risk of cancer of the liver and biliary tract for munitions workers exposed to dinitrotoluene has been reported by US investigators. The six observed cases among the 4989 exposed workers represented rate ratios for hepatobiliary cancer of 2.67 or 3.88 in comparison with either the US population or a group of 7436 unexposed workers at the same plant, respectively. Subjects had worked at the study plant for at least 5 months between 1949 and 1980. No relationship between duration of exposure and increased risk of developing hepatobiliary cancer was observed (Slayet al., Journal of Occupational Medicine 1993, 35, 291).

Antimony
US investigators have suggested that the current occupational exposure limit for antimony is not adequate to prevent skin disease, after reporting the cases of three workers who developed dermatitis following exposure to antimony dust and antimony trioxide fumes. Evaluation of the work process, which included breaking up and melting antimony ingots revealed an 8-hr TWA for antimony of 0.39 mg/m³, although for one 250-min period the average concentration was 0.67 mg/m³. The current UK occupational exposure standard for antimony is 0.5 mg/m³ (8-hr TWA) (White, Jr et al., Journal of Occupational Medicine 1993, 35, 392).

Cancer and parental drug use
An increased incidence in children of the rare cancer rhabdomyosarcoma was associated with parental use of marijuana and cocaine. In a US case-control study comparing 322 cases (aged 0-20 years) with 322 controls, mother’s marijuana use during the year before their child’s birth was associated with a three-fold increased risk of disease, while cocaine use was associated with a 5.1-fold increase. Father’s marijuana or cocaine use were each associated with around two-fold increases (Grußner et al., Cancer Causes and Control 1993, 4, 217).

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