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Review Section

Carcinogenesis and Sanity

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SUMMARY

The evaluation of food from the viewpoint of possible carcinogenic hazard should include a search for ways of reducing the existing human cancer burden. The possibility that carcinogens may be present in natural foods, or find their way into foods as a result of traditional methods of processing, can no longer be ignored. The problem of cancer of the colon in man deserves special consideration in this connexion. Complete avoidance of cancer hazard is impracticable. An upper limit for the contamination of food with benzo[*a*]pyrene should be agreed and enforced. The system of scrutiny of food constituents for safety should be more flexible, so that priority can be given to potentially more serious hazards, whether they be additives or contaminants. A system is suggested for categorizing potential additives prior to testing with regard to possible carcinogenic risk. Urgent attention should be paid to the possibility that nitrosamines and other carcinogens may be formed in foods during processing or cooking by the interaction of ingredients, or as a result of pyrolysis.

More attention should be paid to the mechanism of carcinogenesis by particular agents, especially to the distinction between carcinogenicity and co-carcinogenicity. Although hazard from co-carcinogens cannot be ignored, it may be regarded in a less serious light, to the extent to which the effects of such compounds are reversed after cessation of exposure.

Introduction

The main object of carrying out carcinogenicity tests in laboratory animals on food additives and substances that may contaminate food is to ensure that foodstuffs do not cause cancer in man. In fact, no laboratory test can provide such assurance, since even the most elaborate battery of tests is destined to leave that margin of doubt engendered by inter-species variation. This margin of doubt may be reduced, except in the case of major additives or natural food constituents which constitute more than 1% of the diet, by insistence on a 100-fold safety factor, whereby the permitted concentration of a substance in food is less than 1/100th of a concentration found to be without any observable toxic effect in any animal species. In this context one may note in passing that there is no established definition of either 'observable' or 'toxic effect'. The level of observability depends on the sensitivity of the most sensitive test method available, or on the powers of observation of those responsible for the conduct of the test. The problem of knowing how to distinguish between a toxic effect and a non-toxic effect is not only unresolved but probably unresolvable.

Where a potential food additive is found to be carcinogenic, its use in food is prohibited irrespective of the fact that there is no fully acceptable definition of 'carcinogenicity'. To

date, the legislators have taken no action with regard to co-carcinogenicity. In a situation where definitions are so shaky and the scientific basis for decision is so slippery, it would not have been surprising if the main source of motivation in legislators were the protection of themselves against attack for permitting the use in food of a chemical which subsequently proved to be toxic. In any event, they are requiring food manufacturers to conduct an ever-increasing number of tests on proposed new food additives, even some with the most innocent-looking formulae. One would not necessarily complain about this, however, if problems of possibly far greater importance were not being neglected.

There are only limited facilities, in terms of laboratory space and trained personnel, for long-term toxicological work. Nevertheless, it is probably not at present true that the demand by regulatory authorities for extensive tests on low-risk compounds is holding up research on possibly more important problems. The fact is that, even if the policy on potential new additives became more permissive and less expensive in terms of facilities, there would be no extension of work on problems not affected by existing legislation. Such an extension would require a complete reappraisal by the authorities of the problem of the safety of food in respect of carcinogens and an increase in the provision of government money for more basic research on—for want of a better term—the 'carcinogenic safety' of food.

Even without such a reappraisal, however, the situation is getting out of hand. With regard to long-term tests, the number of new 'low risk' compounds that need to be evaluated is rising rapidly to the point at which the capacity of the available test facilities is exceeded, and already there is a large backlog of substances currently used in food manufacture that have not been fully examined. Unless the regulatory authorities see fit to provide more adequate guidance with regard to priorities, the testing of 'very low risk' compounds will delay tests on more useful or 'higher-risk' substances. The net result is liable to be a serious slowing down not only in the rate of advance of food technology, but also in the discovery of food factors of real importance in relation to carcinogenesis.

It is often said that a knowledge of chemical structure provides no basis for the prediction of carcinogenicity or non-carcinogenicity (Bonser, 1967). Whilst it is true that known carcinogens differ widely in structure, it is possible in practice to predict, sometimes with near certainty, the likelihood of carcinogenicity or non-carcinogenicity in the case of a large number of substances. Part of the confusion in this connexion relates to the failure to consider co-carcinogenicity as part and parcel of the same fundamental problem (see p. 491). At present, carcinogenicity tests are not required in the case of normal body constituents, but whether or not they are required for substances closely related chemically to normal body constituents, or to chemicals belonging to a class for which as a whole there is abundant evidence of non-carcinogenicity, tends too often to depend on the decision or whim of the most suspicious member of the appropriate advisory committee. As long as this individual can claim that the possibility of carcinogenicity cannot be excluded without tests, the chances are that higher committees will endorse his suggestion that such tests are necessary. This sequence of events could be avoided by allocating substances to the following categories, according to the risk that they will prove to be carcinogenic, and by basing priority for carcinogenicity evaluation on the risk-category assigned:

I. Substances that are closely related chemically to known carcinogens or mutagens, that may be expected to react chemically with nucleic acids and proteins, or that may be expected to give rise to metabolites with these properties.

II. Substances belonging to chemical classes not previously investigated for carcinogenicity. Hormones.
III. Substances without obvious toxic effect or without pharmacological activity known to be associated with carcinogens.
IV. Substances closely related to normal body constituents or to a class of compounds for which, as a whole, there is abundant evidence of non-carcinogenicity.
V. Normal body constituents other than hormones.

If this were done, the first duty of advisory committees would be to determine the category into which a previously untested substance should be put; the decision as to the need for a particular substance to be tested, and the urgency of this need, would be a separate exercise. Knowledge of the available facilities, of the likely degree of human exposure and other considerations, apart from the category of risk could influence the latter decision. Under such a system, fear of being blamed for making a wrong decision would be attached only to the possibility of misappraisal of known facts.

Need for reappraisal of the problem of the 'carcinogenic safety' of food

As indicated above, a complete reappraisal of the whole problem of the carcinogenic safety of food is overdue. Apart from the need to use limited test facilities to better advantage, there are three main reasons why this is so:

(i) Research on the carcinogenic safety of proposed new additives and processes is unlikely to throw any light on the existing high incidence of cancer.
(ii) Some of the most potent carcinogens so far found in food occur naturally or come to contaminate food in the course of traditional methods of processing.
(iii) It is no longer possible to ignore the problem of co-carcinogenicity.

Carcinogenicity testing in relation to the existing cancer problem

Theoretically, carcinogens present in food could induce cancer at any site in the body. However, extensive studies on laboratory animals indicate that certain organs and tissues are more at risk than others. Carcinogens of a wide range of chemical class induce cancer in the liver, and those of a somewhat narrower range induce cancer of the gastro-intestinal tract. A still narrower range induces cancer of the lung, mammary gland, kidney or urinary tract. The induction of cancers at other sites by substances administered by mouth is relatively uncommon (Table 1). The most important exception is in relation to the nitrosamines: different compounds of this class induce cancers in a very wide variety of sites and tissues, sometimes irrespective of the route of their administration.

Despite the apparent vulnerability of the liver to the induction of cancer by orally administered carcinogens, it is noteworthy that the incidence of cancer of this organ is low in the United States and Western Europe, where the fullest use is made of synthetic chemicals in the processing of food. Moreover, in these countries, despite the increasing sophistication of food processing, there has been no apparent increase in mortality from liver cancer during the past 60 yr. During the same period, mortality from cancer of the stomach has fallen sharply in the United States (Haenszel, 1958) and less sharply in Britain (Doll, 1967). There has been a moderate increase in mortality from urinary-tract cancer in Britain (Case, 1956) but failure to check certain occupational cancer hazards may be implicated here.

The facts, taken at their face value, do not suggest an urgent need for greater stringency than at present in relation to the testing of food additives. If anything, they tend to show that

Table 1. *Predominant sites of cancer induction following oral administration of known carcinogens of various chemical classes*

Class of compound	Site of cancer induction						
	Liver	Gastro-intestinal tract	Lung	Bladder	Mammary gland	Kidney	Other
Polycyclic aromatic hydrocarbons (e.g. benzo[*a*]pyrene, 3-methylcholanthrene, dibenz[a,h]anthracene, 7,12-dimethyl-benz[a]anthracene)		++ (mainly of forestomach in rodents)	+		++ (by massive doses in rats)		Lymphomas
Heteropolycyclic compounds (e.g. 3,4,5,6-dibenzcarbazole[1*])	+	+					
Aromatic amines and related compound (e.g. 2-aminofluorene, 2-acetylaminofluorene, 2-naphthylamine, benzidine, 4-aminostilbene, 4-aminobiphenyl, 2-nitrofluorene)	+++	+		+++	+++		Ear duct
Azo compounds (*o*-aminoazotoluene, *p*-aminoazobenzene, *p*-dimethyl-aminoazobenzene)	+++			++			
Nitrosamines[2, 3]	+++	+++	++	++	++	++	Brain, lymphomas and many other sites
Urethane and related carbamates[4, 5, 6]	+	+	+++				Angiomas, lymphomas, sebaceous gland (Zymbal) carcinomas and neoplasms at other sites
Hydrazines[7, 8]	+		++				

Nitroquinolines (e.g. 4-nitroquinoline-1-oxide[9, 10])		+ (Tongue and other oral sites)		
Natural products				
Aflatoxin[11, 12, 13, 14]	++	+	+	
Safrole[15]	++			
Pyrrolizidine alkaloids[16]	++			
Griseofulvin[17]	++			
Cycasin[18]	++		+	
Metals				
Selenium[19]	+			
Lead[20, 21]			+	
Radium				Bone
Miscellaneous compounds				
Thiourea and other thyroid suppressants	+			Thyroid
Carbon tetrachloride	++			
Ethionine[22]	++			

* For most of the data summarized there is abundant evidence in the literature and the reader is referred to Hartwell (1951) and Shubik & Hartwell (1957) for specific references. The references given in this table relate to isolated observations and observations made more recently than 1957:

[1] Armstrong & Bonser (1950)
[2] Magee & Schoental (1964)
[3] Druckrey, Preussmann, Ivanković & Schmähl (1967)
[4] Toth, Della Porta & Shubik (1961)
[5] Tannenbaum, Vesselmoritel Maltoni & Mitchell (1962)
[6] Klein (1962)
[7] Biancifiori & Ribacchi (1962)
[8] Biancifiori, Bucciarelli, Clayson & Santilli (1964)
[9] Fujino, Chino & Imai (1965)
[10] Carter, Heathcote & Roe (1967)
[11] Lancaster, Jenkins & Philp (1961)
[12] Le Breton, Frayssinet & Boy (1962)
[13] Salmon & Newberne (1963)
[14] Butler & Barnes (1966)
[15] Homburger, Friedler, Kelly & Russfield (1961)
[16] Barnes & Schoental (1958)
[17] Hurst & Paget (1963)
[18] Laqueur, Mickelsen, Whiting & Kurland (1963)
[19] Nelson, Fitzhugh & Calvery (1943)
[20] van Esch, van Genderen & Vink (1962)
[21] Boyland, Dukes, Grover & Mitchley (1962)
[22] Farber (1963)

higher standards of food hygiene, which depend in part on the proper use of additives, may have led to the greater carcinogenic safety of food, possibly by the elimination of natural carcinogens such as fungal toxins.

Perhaps the most important aspect of the current human cancer problem is the high incidence and mortality from cancer of the colon and rectum. No other species of animal is afflicted with this type of cancer to the same extent, and mortality from it has remained more or less constant during the last 60 yr. If a factor in food is involved in the aetiology of this condition, no amount of research on newly introduced or proposed new additives is likely to reveal its nature. Instead one will have to look at natural food constituents, or at processes 'generally recognized as safe' on account of traditional usage. In this connexion, it may be relevant to point out that a peculiarity of man is that he cooks his food. No other species of animal does so, and no other species has such a high incidence of colonic cancer.

Natural carcinogens, universal carcinogenic contaminants and traditional methods of food preservation

It is perhaps an accident of the natural order of things that benzo[*a*]pyrene, a potent carcinogen of the aromatic polycyclic hydrocarbon class, should have been granted a highly characteristic ultraviolet-absorption spectrum, which enables it to be detected in very low concentrations (Genest & Smith, 1964). Because of the ease with which its presence may be detected, and because it is produced in the course of pyrolysis of a wide variety of organic materials (Gilbert & Lindsey, 1957), benzo[*a*]pyrene has been found in almost every part of the human environment, including a wide variety of foods. The latter include a variety of smoked foods (Bailey & Dungal, 1958; Gorelova & Dikun, 1958a; Dungal, 1961), charcoal-broiled steaks (Lijinsky & Shubik, 1964), bread (*Les Journées Scientifiques*, 1962), and coffee (Chassevent & Héros, 1963). Fallout of benzopyrene-containing particles from the atmosphere leads to the contamination of cereal crops (Galuškinová, 1964) and of drinking water (Borneff, 1964). It is therefore a painful fact of life that man is bound to consume detectable amounts of this known carcinogen. Nevertheless it is surely ironic that the regulatory authorities, who are prepared to devote so much of their time to low-risk hazards, have so far ignored this particular problem.

The most serious risk is associated with the consumption of certain smoked foods, particularly home-smoked as opposed to factory-smoked products (Gorelova & Dikun, 1958b). It is certainly possible, though not proved, that the high mortality from gastric cancer in parts of Iceland is associated with the high proportion of smoked foods in the diet (Wynder, Kinet, Dungal & Mitsuo, 1963; Dungal, 1961). Irrespective of whether benzo[*a*]-pyrene is carcinogenic in man, it is completely illogical not to introduce some control over the extent to which it may be permitted as a contaminant in foods sold to the public.

However this is only a small part of the problem. Almost certainly benzo[*a*]pyrene is only one of a number of carcinogens that are produced during pyrolysis, and therefore in a number of cooking processes. Other carcinogenic polycyclic and heterocyclic compounds and, in some circumstances nitrosamines (Ender, Havre, Helgebostad, Koppang, Madsen & Ceh, 1964), may also be produced.

Knowledge of the concentrations of polycyclic hydrocarbons and nitrosamines in manufactured foods or in foods after cooking, and of the possible dangers of heating mixtures of particular ingredients in the preparation of special dishes is entirely lacking.

In this connexion, the full impact of the discovery that dimethylnitrosamine may be formed during the production of herring meal (Ender *et al.*, 1964) has yet to be felt. The story,

as now pieced together by these workers and others in Norway (Ender *et al.*, 1964; Sakshaug, Sögnen, Hansen & Koppang, 1965; Koppang, 1966; Koppang & Helgebostad, 1966a, b), is as follows. Since the early 1950's it has been the practice in Norway to add a solution of sodium nitrite or a mixture of sodium nitrite and formalin to fish to prevent the conversion of trimethylamine oxide to trimethylamine and dimethylamine. This conversion, which is a feature of autolysis and bacterial decay in fish, may be retarded by the addition of nitrite. However, if nitrite is added after some decomposition has already occurred, the ground is set for the formation of dimethylnitrosamine by reaction of dimethylamine and nitrite under the temperature conditions encountered during the processing of the fish into meal. This sequence of events, it seems, led to outbreaks of toxic hepatitis in sheep, to serious epidemics of fatal hepatosis on mink and fox farms in Norway during the 1957–1961 period (Böhler, 1960, 1962) and to a similar outbreak on fur farms in Britain during 1961. In these outbreaks the principal manifestation of intoxication was hepatosis, and only a few examples of hepatic cancer were encountered in animals which survived for long periods. The acute manifestations of the field cases have been simulated in the laboratory by the deliberate exposure of mink to 2·5 or 5 ppm dimethylnitrosamine (Carter, Percival & Roe, 1968). However, as is well known, dimethylnitrosamine may act as a potent carcinogen in other species (Magee & Barnes, 1956, 1962).

Another potentially alarming report was that by Marquardt and Hedler (1966) who claimed to have found diethylnitrosamine in flour. It was suggested that this was produced during the drying of the grain in a stream of exhaust gases which contained oxides of nitrogen and that the latter reacted with diethylamine in the wheat to produce diethylnitrosamine. Thewlis (1967), testing flours used in the United Kingdom, failed to confirm this finding, but subsequently Hedler & Marquardt (1968) have reported detecting diethylnitrosamine not only in samples of the flours tested by Thewlis (1967) but also in parts of the wheat plant and in the grain, and in milk and cheese. In view of the discrepancy in the findings with flour, further studies are obviously required to settle what must still be regarded as an open question.

The problem of distinguishing between carcinogens and co-carcinogens

Furth & Furth (1936) reported that exposure to ionizing radiation increased the incidence of leukaemia (malignant lymphoma) in mice. A year later Lacassagne (1937) and Gardner (1937) showed independently that administration of oestrogens may have the same effect. Subsequent work indicated that the incidence of lymphoma in mice is also increased by exposure to chemical carcinogens such as those of the polycyclic hydrocarbon type (Morton & Mider, 1938) and urethane (Kawamoto, Ida, Kirschbaum & Taylor, 1958). Taken at their face value, these facts suggest that oestrogens, X-rays, polycyclic hydrocarbons and urethane share a single property—carcinogenicity. Indeed, such evidence would, in most cases, be sufficient for the purposes of the Delaney Amendment, according to which the use of 'carcinogens' as food additives is absolutely prohibited. But knowledge with regard to the mechanism of leukaemogenesis in mice goes further. Adrenalectomy or orchidectomy may also increase lymphoma incidence (Law, 1947) and it now seems that these, and all other stimuli that increase the incidence of lymphoma, have in common the property of giving rise to or perpetuating an anatomically distinguishable condition of the thymus wherein the cortical zone of large immature cells is well developed (Kaplan, 1961, 1966). In the high-lymphoma AK strain of mice this hypertrophy of the cortical zone persists into adult life instead of regressing during infancy as in other strains.

Gross (1951) first demonstrated that murine lymphoma may be induced by an RNA virus (Gross Passage A), and that the virus is naturally transmitted vertically from one generation to the next. Several such viruses have since come to light (Stansly, 1963) and the question arises as to whether a virus of this type is invariably involved in the aetiology of the disease. Though the answer is uncertain, the odds are definitely in favour of the proposition.

It is now known that viruses, such as Gross Passage A virus, may act as helpers by providing the information necessary for the production of the protein coat for yet another virus, the mouse sarcoma virus (Harvey, 1964; Moloney, 1966), though there is no evidence that the mouse sarcoma virus is itself involved in lymphomagenesis.

With regard to the 'induction' of lymphoma in mice by polycyclic hydrocarbons, urethane and oestrogens, is it justifiable to regard all three agents as 'carcinogens' on the evidence quoted above? Surely 'modifiers of carcinogenesis' or 'co-carcinogens' would be more appropriate terms.

A consideration of the published reports of carcinogenesis by combinations of chemical and viral agents (Roe & Rowson, 1968) reveals many other examples of the non-specific enhancement of viral tumorigenesis by chemical agents which are generally regarded, on the basis of evidence from many test systems, either as potent carcinogens or as non-carcinogens. Ahlström & Andrewes (1938) injected rabbits subcutaneously or intramuscularly with 3-methylcholanthrene, benzo[*a*]pyrene or coal tar. In animals so treated, but not in untreated controls, the subcutaneous injection of Shope fibroma virus led to the development of massive local tumours, and its intravenous injection resulted in generalized fibromatosis. The virus could be recovered from the resulting neoplasm in both cases. Later work indicated that the effects of 3-methylcholanthrene, benzo[*a*]pyrene or coal tar could be equally well achieved by exposure to X-rays (Hurst, 1938; Clemmensen, 1939), prednisolone (Bergman, Jonsson & Ahlström, 1962), cortisone (Harel & Constantin, 1954), 6-mecaptopurine (Hurst, 1964) and methotrexate (Allison & Friedman, 1966). These later findings suggest that in the original experiments of Ahlström & Andrewes (1938), methylcholanthrene, benzo[*a*]pyrene and coal tar enhanced carcinogenesis not by a specific effect related to their carcinogenic potential, but non-specifically by an immunosuppressant effect. Recent work has confirmed that carcinogens of the polycyclic hydrocarbon type may suppress both the production of circulating antibodies and cellular mechanisms of immunity (Stjernsward, 1965, 1966; Ball, Sinclair & McCarter, 1966; Weston, 1967).

At present the accepted definition of a carcinogen is concerned not with the mechanism of carcinogenesis, but solely with the overall relationship between cause and effect. Thus, if control animals that are left untreated do not develop tumours whilst comparable animals exposed to a chemical develop tumours, the phenomenon is regarded as carcinogenesis and the chemical agent as a carcinogen. But such a refusal to consider mechanism entails the absurdity illustrated above, that one is led to equate exogenous chemical agents with endogenous hormones, with procedures such as adrenalectomy, and with agents whose essential activity is clearly not tumour induction but immunosuppression.

As pointed out above, those responsible for legislation in relation to the carcinogenic safety of food have so far avoided the problem of co-carcinogens. Lack of a clear concept of co-carcinogenicity is one reason; seeming impracticability is another. The sheer weight of papers concerned with the two-stage mechanism of carcinogenesis has led many to conceive of co-carcinogenesis in the very restricted sense of tumour-promotion. But a more acceptable definition of a co-carcinogen is "an agent which increases the risk, and/or shortens the induction time, of tumorigenesis in response to a carcinogen". Elsewhere (Salaman & Roe,

1964), it has been suggested that the term co-carcinogen should only apply to agents which play a specific and integral part in the genesis of tumours. This would exclude non-specific modification of carcinogenesis by factors such as facilitation of absorption of the true carcinogen into the body or into the cell, or blockage of detoxification or excretion mechanisms. If all such non-specific mechanisms were excluded, would any examples of 'true' co-carcinogenesis remain? This is an open question. In any case, when tumours arise in the course of long-term feeding or injection studies, it is not possible without investigation of the mechanism to distinguish between carcinogenesis and co-carcinogenic enhancement or simple modification of the carcinogenic effect of an agent already in the test system.

In a recent paper, Golberg (1967a) refers to the same problem under the heading "Primary v. secondary effects". If rats are fed high levels of ethylene glycol, or polyoxyethylene (8) monostearate, they excrete enough oxalate for the formation of oxalate bladder stones. The prolonged residence of these in the bladder predisposes to the development of bladder tumours. The evidence that tumours arise in the absence of stone-formation is negligible. It seems therefore that tumour formation is a purely secondary phenomenon. Despite this, polyoxyethylene (8) stearate stands condemned by some as a 'carcinogen'.

It is possible that during the next few years a way of escape from these dilemmas will become available and that the close correlation between *in vitro* 'malignant' transformation of cells and the malignant behaviour of cells *in vivo* will provide the key. At present there is no evidence to suggest that the conversion at the cellular level from the normal state to malignancy can occur in the absence of a change in the nucleic acids of the cell. Where the change is brought about by the incorporation of a virus into, or its addition to, the normal cellular nucleic acids, the correlations between *in vitro* 'malignant' transformation and *in vivo* malignancy are easy to detect, to measure and to define. Such a change involves the addition to cells of a large standard block of 'information' which, if expressed, is 'printed out' in the form of serologically-characteristic new proteins.

Berwald & Sachs (1963, 1965) described *in vitro* transformation in response to benzo[*a*]-pyrene and 3-methylcholanthrene but not to the non-carcinogenic hydrocarbons 8-methyl-benz[*a*]anthracene, chrysene or pyrene. Huberman & Sachs (1966) reported that, in the case of benzo[*a*]pyrene, the number of cells transformed is directly proportional to the concentration applied. Heidelberger & Iype (1967) have partly confirmed these findings. Other workers have had difficulty in doing so, though Sanders & Burford (1967) succeeded in inducing transformation with *N*-nitrosomethylurea. In their system, however, *N*-methylurea, which has not been shown to be carcinogenic, also produced transformations. According to Sachs (1966), unlike cells transformed by a particular virus, cells transformed by a particular chemical agent 'print out' a wide variety of new information in the form of antigenically new proteins. It seems likely therefore that 'malignant' transformation is not itself evidence of a specific change in a cell, and that a variety of alterations in nucleic acid content may bring it about. Chemical agents may cause changes in chromosome number or morphology that are not accompanied by the acquisition of the power to produce tumours when the cells concerned are introduced into animals (Borenfreund, Krim, Sanders, Sternberg & Bendich, 1966), but it is not known whether such changes necessarily render a cell more susceptible to malignant transformation by subsequent exposure to the same or a different agent. Similarly, there are apparently degrees of transformation by viruses that may be brought about in successive stages (MacPherson, 1966; Sabin, 1966).

Clearly, it is not yet possible to devise a method for predicting carcinogenicity in terms of *in vitro* 'malignant' transformation. The positive result obtained by Sanders & Burford

(1967) with *N*-methylurea deserves special attention in terms both of corroboration at the *in vitro* level and of extended examination for carcinogenicity *in vivo*. Similarly, failure to induce *in vitro* transformation by urethane (Berwald & Sachs, 1963, 1965) or by dimethylnitrosamine (Sanders & Burford, 1967) deserves further study. In both these cases failure may be explained by absence from the tissue cultures of enzymes necessary to convert inactive precursors to active metabolites. In other cases, failure could occur if suspect carcinogens were dependent for their activity on the presence of other agents.

Under such circumstances it might be reasonable to regard the agent which effects the change in nucleic acids as the 'carcinogen' and the other agent as the co-carcinogen or 'modifier'. In their review of the literature on carcinogenesis by combinations of viruses and chemical agents, Roe & Rowson (1968) found no example of combined action where it was certain that both agents involved in the induction of a tumour acted by inducing a change in the cellular nucleic acids. In most instances, the most likely hypothesis was that only one agent did so and that the other agent did no more than modify the activity of the first; this was true even where both agents behaved in other systems as potent carcinogens by themselves.

One fact emerges rather clearly from these various considerations. In a world in which a variety of potentially oncogenic viruses abound, and in which there is no escaping background exposure to ionizing radiation or to chemicals known to be capable of inducing *in vitro* transformation, it is impossible, by any simple test for carcinogenesis, to distinguish between true carcinogens, capable of changing the nucleic acid in cells, and co-carcinogens which do no more than facilitate such a change (or the expression of such a change) brought about by another agent already in the system. On the other hand, it is equally evident that it is important to try to make this distinction before the list of compounds to be excluded from our environment becomes so long as to render the task utterly impossible. *A priori*, if the distinction could be made, one would be more concerned about agents that alter nucleic acids than about agents which do not because on present evidence such alterations are liable to be irreversible. But amongst the large number of co-carcinogenic factors some would merit more concern than others. Where hormones influence cancer development the mechanism may involve the 'switching-on' of information (normal or abnormal) that is otherwise suppressed. Such a mechanism of co-carcinogenesis should cause more concern than one that depends on nothing more than non-specific irritation. The effects of co-carcinogens of either the hormonal or irritant types are not necessarily completely reversible (Roe & Clack, 1963; Mühlbock, 1962, 1963). However, since prolonged exposure to such influences is usually required, and since this is often accompanied by warning signs, such as feminization in the case of oestrogens, and hyperaemia and inflammation in the case of irritants, it would be quite unreasonable to regard them as dangerous in the same way as, say, certain nitrosamines which may induce cancer after even single exposures at concentrations which produce no immediate effect.

In practical terms the arguments presented here do not take us much further than to provide a rational basis for insisting that evidence of carcinogenicity must be sought in more than one biological system. If cancer may be induced by an agent in a variety of species and following a variety of routes of administration, then the likelihood is that the agent is a true carcinogen. If cancer can only be induced in one species or under special circumstances, or if the effect observed is only an increase in the incidence of a type of tumour that arises commonly in the test species, or if there is no clear relationship between dose and effect, then the likelihood is that where tumours appear, the agent is acting only as a co-carcinogen.

The theoretical implications are perhaps more impressive. At present, even where the only evidence of carcinogenicity is a marginal increase in a particular type of neoplasm in a single species, the agent responsible falls under a heavy cloud of suspicion with regard to carcinogenicity. One purpose of the present discourse is to explain why at least equal weight should be given to negative evidence derived from properly conducted experiments in other species. To exclude from the human environment agents which happen to fulfil some particular co-carcinogenic requirement for tumour induction in a test system serves no useful purpose. By the same token no animal test system can exclude the possibility that an agent, inactive in the laboratory, can light up a potentially oncogenic virus in man.

In the long run it may be feasible to predict, by chemical analogy, structures that will react with nucleic acids and cause malignant transformation, and to predict by pharmacological and/or chemical analogy structures which are liable to act co-carcinogenically by various mechanisms. This may be the most important justification for the plea for a closer study of the mechanism by which exposure to agents results in increased tumour incidence.

The significance of sarcoma induction at the site of parental injection of food additives

There are, of course, many aspects of the problem of carcinogenesis and food that have not been touched on. Perhaps the most important is the rationale of examining food additives, particularly non-absorbable additives, for carcinogenicity by the technique of subcutaneous injection in rats or mice. However, the polemics of this matter have been recently presented in this journal (Grasso & Golberg, 1966) and elsewhere (Golberg, 1967a, b) and little else need to be said. Perhaps the happiest solution that may be hoped for in relation to this particular problem would be the demonstration that the induction of sarcomas at the site of injection of a substance may be a manifestation of co-carcinogenesis rather than of carcinogenesis.

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