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RESPIRATORY IRRITATION AND DIET AS RISK FACTORS FOR LUNG CANCER

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ABSTRACT

Mutation in the form of persistent DNA damage or chromosomal aberration is crucially implicated in carcinogenesis. However, during recent years more and more examples of substances which increase cancer incidence but which are not mutagenic have been coming to light. A common feature of the toxicity of such substances is that they stimulate cell-replication, either because they are irritants or because they interfere with hormonal or other mechanisms involved in tissue homeostasis. Most DNA damage, however caused, is rapidly and efficiently repaired. However, under conditions of rapid cell replication there is less time for DNA repair between successive cell divisions and therefore an increased risk that unrepaired DNA damage, that is to say mutant DNA, is passed on to subsequent generations of cells. This explains how non genotoxic irritants can increase the risk of carcinogenesis. Mutagens are generated during the metabolism of ordinary foodstuffs, e.g. during the peroxidation of fats. The repair of the DNA damage caused by such endogenous mutagens may also be hampered by exposure to irritants or by other factors, such as hormones or overnutrition, which actively stimulate cell-replication. The contribution of irritation and regenerative hyperplasia to the aetiology of lung cancer is discussed with reference, inter alia, to asbestos, tobacco smoke and certain other chemicals.

INTRODUCTION

At the time when epidemiologists first reported the association between smoking and lung cancer [1,2] it was widely believed by cancer researchers that only a small minority of the large number of chemicals in the world are capable of causing cancer - i.e. are carcinogenic. The idea that irritation per se can, by itself, cause cancer was vigorously rejected, although it was agreed that some chronic irritants can, by a process known as tumour promotion, enhance the risk of cancer development in response to prior exposure to a known carcinogen. Tumour promotion was the name given to the second stage of a theoretical two stage process [3,4,5] the first stage of which (tumour initiation) was thought to take the form of a mutation in cellular DNA.

The two stage theory became very popular despite the fact that mathematical modelling strongly suggested that more than two stages are involved and despite the fact that none of the premises on which it was founded proved to be wholly true. According to the theory, exposure to non-mutagenic irritants alone should not give rise to cancers. However, all the

irritants which have been used as tumour promoters in the mouse skin or other tissues (e.g. urinary bladder [6], forestomach [7], liver [8]) have been found to be complete carcinogens under conditions of prolonged exposure. In the case of mouse skin, this is true for the chemical of plant origin, 12-0-tetradecanoyl-phorbol-13-acetate (TPA), that has been very extensively studied [9].

Throughout the last 40 or so years some investigators have been so enchanted by the simplicity of the two-stage carcinogenesis paradigm [10] that, rather than question the quality of the data on which it was founded, they have sought alternative explanations of experimental data which clearly challenge it. However, during the last year or so the picture has radically changed with the realization of three facts. First, mutagens are not only abundantly present in the environment, but are produced endogenously during the metabolism of simple foodstuffs - particularly during the peroxidation of fats [11]. Secondly, most of the damage to DNA caused by either exogenous or endogenous mutagens is rapidly and effectively repaired before the cells in which it has occurred are required to replicate [12]. Thirdly, if, for whatever reason, cells are required to replicate before DNA-repair is complete there is a danger that cells with abnormal DNA, i.e. mutant cells, will form clones of cells which may be cancerous in nature [13]. Herein lies a wholly new reason for being concerned about non-genotoxic chemicals which stimulate cellular replication and hyperplasia.

It is against this background that we need now to consider the possible roles of respiratory irritation and diet as risk factors for lung cancer.

ASBESTOS

Obsessed with need to correlate carcinogenicity with mutagenicity, numerous investigators have endeavoured to show that asbestos fibres are mutagenic. Clastogenic effects have been reported [14] but no convincing evidence of mutagenic activity. Nevertheless the inhalation of asbestos fibres, particularly of crocidolite or amosite fibres, is highly associated with increased risk of lung cancer development. Thus according to Buchanan [15], in England and Wales during the period 1961, no less than 54.5% of men and 22.2% of women who died with asbestosis also had either carcinoma of the lung or mesothelioma. A feature of asbestosis is, of course, fibrosis of the lung parenchyma, particularly of the lower lobes where inhaled fibres are most heavily deposited and trapped. According to Whitwell *et al.* [16] many of the lung cancers associated with asbestosis are of the adenocarcinomatous variety and many of them arise in the peripheral lung tissue in areas of intense fibrosis. However, there is also an association between exposure to asbestos fibres and more centrally arising lung cancers of types other than adenocarcinoma. Selikoff and his colleagues [17], in the light of data from a study of insulation workers, concluded that the risks of

carcinoma of the lung associated with (a) asbestos and (b) cigarette smoking are multiplicative as distinct from additive. But, the calculations on which this conclusion was drawn were based on very few cases of lung cancer in non-smoking asbestos exposed workers and on virtually no non-smoking, non asbestos exposed workers. For this reason a simple additive, as distinct from multiplicative, intereaction cannot be excluded. A multiplicative interaction for centrally-arising, non-adenocarcinomatous primary lung cancers was rendered more plausible by the results of e.m. studies in which the impaling of bronchial epithelial cells by asbestos fibres and the accumulation of fibres in the basal cell layer of airway epithelium were observed [18]. Unfortunately, whereas for exposure to cigarette smoke alone, there is considerable information about the occurrence of hyperplastic and metaplastic changes prior to the appearance of cancers in airway epithelium [19], there is no comparable information in relation to exposure to asbestos alone or to combined exposure to both asbestos and cigarette smoke. Laboratory studies have, so far, failed to indicate any synergism between asbestos and cigarette smoke in relation to the type or severity of asbestotic lesions [20].

It is now generally agreed that the long thin shape of asbestos fibres, particularly that of the amphiboles, is an important factor because, despite their length, such fibres can behave aerodynamically like spheres of the same diameter as their cross sections. For this reason large particles, which, if they had been spherical, would have been filtered out in the nose or deposited in the large airways can be carried deeply into the lung. The insolubility, or poor solubility, of the fibres in body fluids is also generally regarded as contributing to the problem. The combination of deep penetration by large particles and poor solubility creates a situation with which the lung is not adequately equipped to deal. When insoluble small particles are deposited in the lung they are phagocytosed by lung macrophages which then carry them, via the lymphatic system, to bronchial lymph nodes or, via the cilial esclator, to the larynx where they are then swallowed and thereafter lost from the body via the faeces. However, the long thin asbestos fibres are too big to the phagocytosed by macrophages, many of which die in the attempt to engulf them. The death of macrophages leads to the release of proteolytic enzymes, tissue destruction, inflammation and reparative cellular proliferation. In the light of what we now know about the association between cellular proliferation and increased risk of mutagenesis generally, the most likely explanation of the carcinogenic risk from asbestos is that it is a form of non-genotoxic carcinogensis in which DNA damage occurs as a secondary event following irritation and hyperplasia. The DNA damage is probably, for the main part, caused by endogenous mutagens. However, the activity of endogenous mutagens may be supplemented by that of inhaled environmental mutagens such as those presënt in cooking and heating fumes, diesel exhaust and tobacco-smoke, etc.

Support for this theory of the mechanism of lung carcinogenesis by

asbestos comes from two sources. First, the demonstration of an inverse relationship between lung clearance rates and fibre length [18] and secondly, cancer risk is seemingly higher for amphibole types of asbestos (e.g. crocidolite, amosite) which are less soluble in tissue fluids than for chrysotile [22].

Other theories to explain asbestos-induced carcinogenesis have been considered in the past. For instance, the role of contamination of asbestos by carcinogenic polycyclic aromatic hydrocarbons adsorbed from the jute sacks in which it was traditionally stored and transported was considered [20]. Skin tumours were produced in mice by applying mineral oils used in the processing of jute [24]. However, it did not prove possible to abolish the carcinogenic activity of asbestos by removing the adsorbed carcinogens. Furthermore, miners exposed to asbestos dust which had not been contaminated with jute batching oils were clearly not free from cancer risk.

TOBACCO SMOKE

When in 1953, Wynder *et al* [25] reported the induction of skin cancers in mice by the repeated application of tobacco smoke condensate, it was widely assumed that the association between smoking and increased lung cancer risk was due to the presence of genotoxic carcinogens in tobacco smoke. It was further thought likely that one or more carcinogenic polycyclic aromatic hydrocarbons, such as 3,4-benzpyrene, were likely to be the culprits. This led to the hope that smoking could be made safe by eliminating such substances from tobacco smoke. It might be possible to do this by modification of the tobacco, by filtration, or by changing the conditions in which the combusion/pyrolysis of tobacco takes place during the course of smoking. Secondly, insofar as it was only a minority of smokers who develop lung cancer, it was postulated that among the heterogenous human population it may be that only a minority are genetically susceptible to carcinogens of the type present in smoke. During the course of the 25 years or so following the publication of the paper by Wynder *et al.* [25], it was shown that most but not all the carcinogenic activity of tobacco smoke condensate for mouse skin was associated with only a small fraction of the condensate [26]. However, no way of selectively removing this fraction, or of preventing its formation, was found. Furthermore, there was seemingly nothing unique about the chemicals in the active fraction: the array of them was similar to that found in smoke derived from the pyrolysis/combustion of other types of organic matter [27] (e.g. coal, wood, bonfire smoke). In one study, the potency of tobacco smoke condensates was found to be far less than that of the particulate matter present in urban air polluted by chimney smoke from the pyrolysis/combustion of coal and by the exhaust fumes of petrol and diesel-powered motor vehicles [28]

The only really unique aspect of tobacco as an organic 'fuel' is its

content of nicotine and related alkaloids. These latter substances can be converted during the curing of tobacco, during its pyrolysis, and within the body of smokers to carcinogenic nitrosamines [29]. However, whether such substances are present in high enough amounts to explain the association between smoking and increased risk of lung cancer is dubious [30].

The possibility that irritants present in smoke may contribute to its carcinogenicity has from time to time been postulated. As early as 1958 Gellhorn [31] concluded that tobacco smoke is more potent as a co-carcinogen than as a complete carcinogen. In other words he was suggesting that the main effect of tobacco smoke was attributable to its irritancy rather than to its content of genotoxic carcinogens. Later my colleagues and I [32] reported that the fraction of tobacco smoke condensate which contains the irritant, hyperplasia-producing, phenolic constituents of smoke actively enhances the skin-tumour inducing activity of known carcinogens.

A serious limitation of all the early experimental studies in the field of tobacco smoke carcinogenesis is that they relied upon the use of the mouse-skin painting model. The biological activity of any irritant gases in smoke could not be investigated using this model. Later investigators attempted to produce lung tumours in laboratory animals by exposing them to tobacco smoke by the inhalation route. The results of such studies were either negative, weakly positive or equivocal [33,34 ,35] as far as the induction of lung tumours is concerned, although in hamsters smoke exposure was associated with the development of cancers of the larynx [36]. Certainly no quantitatively reliable animal model involving exposure by inhalation emerged from these studies. Consequently, it has not been possible to assess the roles of increased cell turnover within the epithelium of the respiratory tract and of epithelial hyperplasia as determinants of lung cancer risk from tobacco smoke.

On the assumption that smokers smoke primarily for nicotine while the adverse effects of smoking on the lung are primarily due to other smoke constituents, there has been consistent pressure for cigarette manufacturers to reduce the tar delivery of cigarettes, e.g. by different blending, by smoke dilution or by filtration. The delivery of nicotine, though generally lower, has been partly maintained by using tobacco with a higher nicotine content. The net result is that during the last 20 years or so cigarette smokers have been inhaling much less tar but only moderately less nicotine. The benefits of these changes in cigarette design on evidence of irritation and hyperplasia of the respiratory tract epithelium are evident from a paper by Auerbach and his colleagues, published in 1979 [37]. It remains uncertain, however, whether it is the reduction in exposure to the irritants or the reduction in exposure to the genotoxic carcinogens present in cigarette tar which is responsible for the now rapidly falling incidence in the age standardized risk from lung cancer in countries such as the United Kingdom [38].

Among the irritants present in tobacco smoke are various aldehydes, including acrolein, acetaldehyde and formaldehyde, and oxides of nitrogen,

including nitric oxide and nitrogen dioxide. It is possible that these irritants contribute to the increased risk of lung cancer in smokers by giving rise to increased rates of cell replication in the epithelium of the lower respiratory tract. Indeed it is possible that the effects of such irritants are far more important than those of the low levels of genotoxic carcinogens known to be present in smoke [39]. Thus, the position may be similar to that for the induction of nasal tumours by formaldehyde in rats. Formaldehyde is genotoxic but when rats are exposed to concentrations of formaldehyde too low to cause necrosis and regenerative hyperplasia of the nasal epithelium no nasal neoplasms arise. Only when exposure levels are increased to the point of causing regenerative hyperplasia does a risk of nasal neoplasia become evident [40].

In the light of these considerations it is perhaps overdue to take a renewed interest in the possible contribution of irritation to the risk of lung cancer associated with smoking.

IRRITATION BY OTHER INHALED IRRITANTS

The possible roles of persistent inflammation, macrophage overloading and increased cell proliferation in the development of neoplasms of the lung in response to titanium dioxide [41] and diesel exhaust particles [42,43] has been discussed by Grasso *et al.* [44].

DIET

There are three ways in which diet may influence the risk of lung cancer development. First, dietary constituents, such as fat, may increase the risk of lung cancer; secondly, dietary constituents may protect against the development of lung cancer, and thirdly, there may be a relationship between the daily intake of calories and the risk of developing all forms of cancer including cancer of the lung.

Evidence for the first of these possibilities was reported by Wynder *et al* [45]. In a comparison of 43 different countries, these investigators found that, after taking account of tobacco usage, there was a highly significant correlation ($p < 0.0001$) between calorie intake from dietary fat and lung cancer mortality. Later Mettlin [46], in a study of the lifestyle of 569 lung cancer patients, found that, after correcting for smoking history and intake of Vitamin A from vegetables, subjects reporting the consumption of whole milk 3 or more times a day had a 2.14 relative risk (RR) of developing lung cancer compared with those who said they never drink whole milk. By contrast the drinking of reduced-fat milk was protective (RR = 0.54).

With regard to factors which protect against the development of lung cancer, most attention has been paid to beta-carotene and to Vitamin A. Peto *et al. [47]* reviewed the epidemiological evidence for protection by these

agents against the development of lung cancer. A likely mechanism of action relates to the efficiency of beta-carotene for quenching the excitation energy of singlet oxygen and for trapping certain organic free radicals. If this is the mechanism of action, then one would expect beta-carotene to reduce the risk of mutation in the epithelium of the respiratory tract when there is exposure to irritants.

In laboratory animals it is possible not only to compare the effects of different diets but also to control the intake of calories. The results of such research suggest that many of the apparent adverse effects of high fat intake can be explained on the basis of high calorie intake [48]. In other words, provided that the daily caloric intake is restricted then the relative contributions of fat, carbohydrate and protein are not important. In a recently completed study of 30 months duration [49] we saw significantly ($p<0.01$) lower incidences of pulmonary adenomas and carcinomas in rats which, from 13 weeks after weaning until the end of the experiment, had their calorie intake restricted to 80% of that consumed by animals given free access to food, compared with ad libitum-fed rats (see Table 1). Earlier, many investigators including Conybeare [50] have reported that calorie restriction reduces the incidence of lung tumours in mice (see Table 2).

Sacher [51] suggested that food restriction retards the ageing process by reducing the metabolic rate. If this is true, then it would mean that the rate at which DNA-damaging electrophiles are generated endogenously is reduced. Masoro [48], however, could not confirm that food restriction reduced metabolic rate.

An alternative theory is that calorie restriction reduces the rate of cell turnover in some or all tissues. Lok *et al.* [52] recently published evidence in support of this theory (see table 3) and this is the explanation favoured by Cohen and Ellwein [54] in their classical 1990 paper. No doubt Lok *et al.* [52] would have found a similar effect of calorie restriction on the epithelium of the respiratory tract had they looked for it.

Table 1. Lung tumour incidence in rats fed on the same diet either ad libitum (AL) or restricted to 80% of ad libitum (R) from 13 weeks post-weaning+ [49].

	AL		R	
Sex	M	F	M	F
No. of rats	150	150	100	100
No. with pulmonary adenoma or adenocarcinoma	7	6	0	0
(%)	4.33		0**	
No. with pulmonary adenocarcinoma	2	2	0	0
(%)	1.33		0	

+ None of the rats were deliberately exposed to any known carcinogen
** Significantly different from AL : $p<0.01$

Table 2. Lung tumour incidence in mice fed 41B or PR diet either ad libitum (AL) or restricted to 75% of ad libitum (R) from 1 week post-weaning+ [50].

	AL		R	
Sex	M	F	M	F
No. of mice	154	15	159	159
No. with pulmonary adenoma or adenocarcinoma	30	24	19*	8***

\+ None of the mice were deliberately exposed to any known carcinogen
* $p<0.05$
*** $p<0.001$

Table 3. Effect of calorie restriction (R) to 75% of ad libitum (AL) food intake on [^{3}H] thymidine-labelling in various tissues of the mouse (from Lok et al. [52]).

Tissue	[^{3}H] thymidine-labelling index		
	AL	R	% inhibition
Mammary gland	0.32 ±0.09+	0.09±0.04	72**
Urinary bladder epithelium	0.74±0.10	0.42±0.11	43*
Dermis	0.91±0.06	0.39±0.03	57**
Oesophageal epithelium	2.67±0.11	1.36±0.12	49**
Crypt cells			
- colo-rectum	8.33±0.59	3.82±0.38	54**
- jejunum	26.8±1.0	17.8±0.7	34**
- duodenum	26.8±1.0	18.1±0.4	29**

\+ Mean ± S.E.
* $p<0.05$ for data analysed on log scale
** $p<0.01$ for data analysed on log scale
*** $p<0.001$ for data analysed on log scale

CONCLUSIONS

In stressing the probable importance of irritation and increased cell turnover in relation to lung carcinogenesis, I would not wish to imply that these factors play any crucial role in carcinogenesis by potent genotoxic lung carcinogens such as bischloromethyl ether [53]. It is in relation to lung carcinogenesis by non-genotoxic agents that their importance mainly lies. Thus, the lung carcinogenicity of materials such as asbestos and titanium oxide can plausibility be explained.

Some chemicals, such as formaldehyde and acetaldehyde, are both genotoxic and irritant. However, the genotoxicity seems not to be potent enough by itself to increase the risk of cancer development. For the latter to occur there has also to be irritancy sufficient to cause cell death followed by regenerative cell replication. There is good evidence that this is the situation with regard to the induction of nasal tumours by formaldehyde in rats.

Although the association between smoking and lung cancer is strong, tobacco is a far weaker mouse skin carcinogen than, say, the suspended particulate matter in polluted urban air. On the other hand, tobacco smoke contains many respiratory irritants, particularly in the vapour phase which cannot be tested for carcinogenicity using the mouse skin model. Thus, the question arises, is it the irritants in tobacco smoke rather than low levels of genotoxins that is important? Furthermore, the realization that non genotoxic irritants may play a role in lung carcinogenesis is clearly relevant to future research on many other aspects of indoor air quality.

Finally, in the light of the results of recent epidemiological and laboratory research, the possibility that dietary factors - both qualitative and quantitative - influence the risk of developing lung cancer needs to be taken more seriously than in the past.

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