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The significance of irritation in respiratory diseases

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F J C Roe

19 Marryat Road
Wimbledon Common
London SW19 5BB
England

ABSTRACT

The term "irritation" means different things in different contexts and to experts in different branches of medicine. Consequently, trivial short-term manifestations which have only social implications are in danger of being confused with manifestations of irritation which are of serious concern for chest physicians, pathologists and epidemiologists. This paper discusses the meaning and significance of irritation in relation to various forms of air pollution.

INTRODUCTION

"..... as healthy tissue and cancer are living facts, will you tell me if you know or can find out how one can slip down into the other, and whether or how it can be prevented from doing so; for that is what concerns a minister of public health. And please do not tell me what happens to mutilated dogs and guinea pigs and starved rats and mice as I have to deal with what happens to unmutilated citizens who suffer these changes whilst others who eat and drink the same things remain unchanged."

(George Bernard Shaw in an Essay on the Collective Biologist)

Irritation of the respiratory tract may be classified according to clinical signs and symptoms, causes or pathological changes. However, the picture is confused by the fact that quite different diseases with quite different causes give rise to similar spectra of symptoms. This makes it difficult to assess the contribution of individual irritants in polluted air as determinants of disease entities such as bronchitis, emphysema, asthma and lung cancer.

It used to be assumed that the inhalation of irritant particles and gases predisposed to chronic bronchitis and emphysema whereas the risk of lung cancer is only increased by the inhalation of chemical carcinogens. The latter assumption stimulated cancer researchers to try to identify carcinogens in polluted air, tobacco smoke and workplaces whilst taking much less interest in the overall irritancy of the atmospheres they were investigating.

Furthermore, since cancer cells are derived from normal cells as a consequence of genetic damage, the additional assumption was made that tests of airborne chemical pollutants for mutagenic activity is a reliable short-cut to the identification of lung carcinogens.

During recent years, our understanding of the mechanisms involved in carcinogenesis has changed. It is now clear that mutagens are generated endogenously during the metabolic processes involved in the conversion of ordinary foodstuffs to energy, and that the DNA of each normal body cell is damaged many times each day by these mutagens. DNA repair enzymes exist to repair most of this damage but are prevented from doing so if cells divide to form daughter cells too soon after the DNA damage occurs (Ames and Gold, 1990). Thus, in the absence of any exposure to exogenous mutagens, frequent cell division as occurs, for example, in response to tissue damage by irritants, which is followed by regenerative hyperplasia, can lead to the accumulation of unrepaired DNA damage (ie. mutations).

Although these new insights into the mechanisms involved in carcinogenesis have mainly stemmed from research not directly concerned with diseases of the respiratory tract, there have long been pointers to the fact that the earlier simplistic theories cannot explain the facts. Thus the high risk of lung cancer associated with the inhalation of asbestos dust does not match the impossibility of demonstrating convincingly that asbestos has mutagenic activity. The same is true for calcium chromate and for cadmium chloride, though seemingly not for the extremely potent lung carcinogen, bischloromethylether (BCME) (Leong *et al*, 1971).

Another reason for not ignoring the likely role of irritation in the causation of lung cancer is the fact that most lung cancers arise, not in otherwise normal lungs, but in lungs showing evidence of chronic inflammatory and/or chronic obstructive lung disease (eg. in the forms of chronic bronchitis and emphysema). Thus, before the Clean Air Act came into operation in the UK in the 1950s, higher rates of both lung cancer and chronic bronchitis in urban areas than in rural areas co-existed. Also, today, the heavy and prolonged exposure of women to cooking and heating fumes in small unventilated kitchens in undeveloped countries is associated with high rates of both chronic obstructive lung disease and lung cancer (Lancet, 1990).

Although not directly relevant to the inhalation of irritants, a particularly striking example of chronic irritation by a non-mutagenic factor giving rise to cancer is provided by feeding studies in rats. Several investigators (Robinson, 1985; Madsen, 1989; Roe *et al*, 1995) have reported the occurrence of squamous carcinomas of the gums and palates of rats as a consequence of the presence in food of spiky fragments of fibrous chaff which penetrate the epithelium and set up foreign body reactions, abscesses, necrosis and regenerative hyperplasia.

ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS: LABORATORY ANIMALS VERSUS MAN

The lung

According to Sorokin (1970), there are well over 40 distinctive types of cell in the normal mammalian lung: 17 epithelial types, 12 connective tissue, bone and cartilage, 8 blood vessel endothelial and 7 muscle or neural. Disease processes, including those attributed to irritation, variously affect these different types of cell.

Laboratory animal experiments have thrown some light on what does and what does not predispose to lung cancer, but major anatomical and physiological differences between man and species such as the mouse, the rat and the hamster limit the value of the latter as models for man (Table 1).

Because of these differences, only limited reliance can be put on rodent studies in relation to evaluating chemicals for irritancy to the respiratory tract. Furthermore, if rodents are used for this purpose, it is essential that evidence of irritation is sought not only in the lower respiratory tract (ie. the trachea and lungs) but also in the nose and larynx. Thus, when Dontenwill *et al* (1973) exposed hamsters repeatedly to inhaled tobacco smoke, they showed pathological evidence of marked irritation and neoplasia in the larynx but only minor changes in the lungs, and when Swenburg *et al* (1980) exposed rats and mice to formaldehyde they saw evidence of irritation and neoplasia in the nose and larynx, but again, very little in the lungs.

The nasal cavity

The anatomy of the nasal cavity is complex in all species. Thus, in different parts of the nose, one finds four different types of epithelium - squamous, ciliated-respiratory, non-ciliated cuboidal/transitional and olfactory - and these different types respond differently to irritants. In rats, hyperplasia and squamous metaplasia of the respiratory epithelium of the nasal turbinates is the commonest response. However, exposure to specific irritants can lead selectively to hyperplastic, atrophic, metaplastic, and/or neoplastic changes in any part of the nose, including the sub-epithelial mucous glands. Amazingly, the presence of inflammation, hyperplasia, necrosis or even neoplasia in a rat may go unobserved unless a careful and systematic histopathological examination of the nose is carried out post-mortem (Young, 1981). In the past, it was not a routine practice to prepare sections of the nasal cavity of laboratory rodents in chronic toxicity/carcinogenicity tests even in studies where exposure was by the inhalation route. Consequently, we know far less about the effects of respiratory irritants in rodents than we should.

An insight into the complexities of studying the effects of inhaled irritants was provided by Johnson *et al* (1990) in their account of the effects of ozone on rat nasal epithelia. These investigators found that the normal DNA replication

rate is 40 times higher in squamous epithelium than that in ciliated-respiratory, transitional or olfactory epithelium, and that exposure to ozone results, selectively, in a marked increase in DNA replication in transitional epithelium and a marked decrease in DNA replication in squamous epithelium.

MECHANISMS INVOLVED IN IRRITATION AND INFLAMMATION

Both chemicals and infectious agents can cause necrosis of the epithelium and/or supporting connective tissue of the respiratory tract. Necrosis of epithelial cells is normally associated not only with ensuing regenerative hyperplasia, but also with inflammatory infiltration by phagocytic cells which take responsibility for clearing the dead cells away. In the course of carrying out this task, they produce proteolytic enzymes which not only break up the dead cells but which may also adversely affect living cells and supporting connective tissue structures, including elastic fibres. Chronic inflammatory infiltration may therefore be associated with the progressive destruction of lung tissue and, particularly, with the loss of lung elasticity. These losses constitute the disease "emphysema". Protection against the proteolytic enzymes produced by inflammatory cells is provided by antiproteolytic enzymes in genetically normal persons, but in the genetically-determined condition known as alpha-1-antitrypsin deficiency, exposure to respiratory irritants such as nitrogen dioxide, predisposes to rapidly progressive destructive lung disease and premature death. There is no documented evidence that alpha-1-antitrypsin deficiency *per se* increases the risk of development of lung cancer unless there is exposure not only to irritants but also to genotoxic agents.

THE EFFECTS OF SOME INHALED IRRITANTS

Formaldehyde

Every pathologist who worked in an autopsy room or who was involved in the processing of specimens for examination under the microscope in the 1950s or 1960s knows from personal experience of the irritative properties of formaldehyde. Exposure led to lachrymation, sneezing, shortness of breath, tightness of the chest and excessive phlegm, but the possibility of cancer risk was not entertained. In the 1960s and 1970s, formaldehyde was reported to be mutagenic for some strains of bacteria and yeast - particularly those deficient in DNA repair enzymes - and to cause reparable DNA-protein crosslinks and chromosomal aberration (IARC, 1982). A test for carcinogenicity of inhaled formaldehyde in mice conducted in the early 1960s (Horton *et al*, 1963) provided evidence of irritation in the trachae and bronchi but no evidence of carcinogenicity. However, in this experiment the nasal epithelium was not examined. Against this background, the report in 1980 of a high incidence of squamous cell carcinomas of the nasal epithelium in rats exposed to 14.3 ppm formaldehyde vapour during 30 hours per week for up

to 2 years (Swenburg *et al*, 1980) came as both a bombshell and a watershed in our understanding of mechanisms of carcinogenesis. Here was evidence of carcinogenicity by a substance which is extensively used in agriculture and in the manufacture of plastics and resins, as an intermediate in many manufacturing processes, and as a fumigant, disinfectant or preservative in cosmetic and medical products, etc. The steepness of the dose-response relationship for carcinogenicity by formaldehyde in the nose of the rat (see Table 1), conflicted with the view held by many that such relationships are linear. Finally, the fact that formaldehyde is known to be produced endogenously as a metabolite in mammalian systems (National Research Council, 1980) conflicted with the widely held view that only man-made and not naturally-occurring chemicals can act as carcinogens.

The most likely explanation of the steepness of the dose response relationship is that put forward by Woutersen *et al* (1986). In rats, exposure to high concentrations of formaldehyde leads not only to reparable DNA damage but also to necrosis of the epithelium of the anterior part of the nose. Repeated waves of necrosis are followed by repeated waves of regenerative hyperplasia associated with a high mitotic rate. Rapid cell turnover reduces the time available for DNA repair between cell divisions with the consequence that mutations are more likely to remain unrepaired before being passed on to daughters cells. Thus, it is that repeated waves of necrosis and regenerative hyperplasia which can result in the accumulation of unrepaired DNA damage, due mainly to the genotoxicity of formaldehyde. Eventually, cancers develop as a consequence of this accumulation of DNA damage. However, if the concentration of formaldehyde is below the threshold required for the production of necrosis and regenerative hyperplasia, then there is time for genotoxic damage caused by formaldehyde to be repaired before cells divide. In these circumstances, the risk of cancer is not increased.

Curiously, under the same experimental conditions, mice proved to be far less susceptible than rats to nasal carcinogenesis by formaldehyde (Kerns *et al*, 1983). The probable explanation of this is that mice are able to respond to being exposed to inhaled irritants by becoming quiescent and reducing the amount of air they inhale. By this means, it seems, they reduce the risks of necrosis and regenerative hyperplasia. What then are the carcinogenic and other respiratory health risks from formaldehyde in man? Levine *et al* (1984) found no evidence of any adverse effect of exposure to formaldehyde on lung function or incidence of chronic bronchitis among morticians, and no clear evidence of any increased risk of cancer of the respiratory tract among persons exposed occupationally to formaldehyde has been found in other epidemiological studies. The conclusion reached by an Ad Hoc Panel on the health aspects of formaldehyde (1988) was that if there is any risk of lung cancer, it is probably very small.

Acetaldehyde

Acetaldehyde is present in various foodstuffs, and is formed endogenously during intracellular oxidation of ethanol. Like formaldehyde, acetaldehyde is a respiratory tract irritant and has mutagenic activity (IARC, 1985). Though less irritant than formaldehyde, exposure to high concentrations of acetaldehyde has been reported to induce cancers of the nose and larynx in hamsters.

One may deduce from these findings with formaldehyde and acetaldehyde that mutagenic activity by itself is not necessarily associated with increased cancer risk, but that, in combination with irritation sufficient to give rise to regenerative hyperplasia, it may be so.

Chlorine

Chlorine is not genotoxic, but is very irritant to the respiratory passages. In a recently reported study, in which mice and rats were exposed to chlorine at concentrations of up to 2.5 ppm for two years, the investigators observed evidence of marked irritation of the nasal cavity - particularly of the anterior part of the nose - but no tumours anywhere in the respiratory tract (Wolf *et al*, 1995).

Nitrogen dioxide (NO_2)

Prolonged exposure of rats to 25 ppm NO_2 leads to enlargement of the lungs with loss of elastic recoil, ie. changes that are associated with emphysema. On the other hand, prolonged exposure to only 2 ppm NO_2, a level which is much higher than that encountered in polluted urban air, gives rise to no more than temporary loss of cilia and focal hyperplasia/metaplasia of the terminal bronchiolar epithelium in rats (Kleineman and Wright, 1961). However, according to Last and Warren (1987), NO_2 and sulphur dioxide act synergistically as irritants for the lung. Hence, in assessing the significance for health of NO_2-pollution of air, one may need to take into account the concentration not only of NO_2 itself but also that of the cocktail of irritants usually found present in polluted air. Fischer *et al* (1985) found no evidence that the NO_2-content of environmental tobacco smoke (ETS) adversely affects lung function in non-smoking women. However, Tunnicliffe *et al* (1994) have recently reported that exposure to 400 ppb NO_2 potentiated the specific airway response of patients with mild asthma.

Ozone

During the summer months in temperate regions, anticyclonic weather conditions favour the formation of ozone from oxides of nitrogen such as are abundantly present in vehicular exhaust fumes.

Exposure to ozone increased airway resistance and has been found to cause proliferative changes in the nasal cavities of rodents (Johnson *et al*, 1990), epithelial changes in the terminal bronchioles of rodents (Barry *et al*, 1988), inflammation and neutrophil activation in humans (Devlin *et al*, 1991), increased airway responsiveness to inhaled allergens (Molfino *et al*, 1991) and in the long term, pulmonary fibrosis in rats (Last *et al*, 1984).

Small particles: PM10

It used to be thought that large airborne particles, unless they are long and thin (eg. crocidolite asbestos), are not damaging to the lungs because they are effectively filtered off by the nose and that small airborne particles are not dangerous because they are not deposited on the lungs, but simply exhaled. This latter belief is now questioned by the results of research on so-called PM10 particles, which have diameters in the nanometer range. Such particles may be present in large numbers (eg. 150,000 per ml) in polluted urban air, and may remain suspended in it for prolonged periods. After inhalation, instead of being simply exhaled, they may be deposited as a consequence of Brownian movement. Several epidemiologists have reported associations between concentrations of PM10 particles and mortality from respiratory and cardiac disease while experimentalists have shown that rats exposed to PM10 develop severe pulmonary inflammation (Seaton *et al*, 1995; Ferrin *et al*, 1990).

IRRITANTS IN INDOOR AIR

Climate and wealth greatly influence the quality of indoor air and exposure to indoor air pollutants. In poor, hot countries, the quality of indoor air is mainly determined by the quality of the external air insofar as ventilation is via open windows and doors. This means that in big cities, industrial airborne pollutants and vehicle exhaust fumes are important sources of irritants in indoor air. However, in poor, cold or intermittently cold climates, cooking and heating fumes in small unventilated dwellings are the main sources of respiratory irritants.

Incomplete combustion of organic fuels, ie. pyrolysis leads to the formation of a wide variety of chemicals, many of which are irritants (eg. various aldehydes, nitrogen dioxide) and some of which are carcinogenic (eg. benzo(a)pyrene and other polycyclic and heterocyclic aromatic compounds). High biomass fuels (eg. animal dung) produce more airborne pollutants per unit of heat than low biomass fuels (eg. natural gas). However, from a qualitative viewpoint, the spectra of pollutants produced are similar for all organic fuels. The most serious immediate danger from exposure to indoor air pollutants derived from the incomplete combustion of cooking and heating fuels is brain damage or death from carbon monoxide poisoning. The longer term dangers are chronic bronchitis, emphysema and lung cancer. Whether the carcinogenic air pollutants are by themselves sufficient to cause lung

cancer or whether lung cancers arise because of combined exposure to carcinogens plus chronic irritants, is not known. All that can be said is that since lung cancers usually arise in lungs showing evidence of prior or co-existent chronic inflammation, it is likely that exposure to irritants contributes to the risk of lung cancer development.

In countries with cold or temperate climates that are wealthy enough to use electricity, low biomass fuels and/or central heating system, and to have houses in which cooking areas are adequately ventilated, environmental tobacco smoke (ETS) can be the most important contributor to indoor air pollution. Smoking involves the incomplete combustion of organic materials and the spectrum of chemicals present in tobacco smoke is similar to that in smoke from wood or coal fires except that it contains nicotine and related alkaloids. Nicotine is an irritant, but by far the biggest contributor to the irritancy of ETS is its contents of aldehydes, including acrolein, acetaldehyde and formaldehyde. In this latter respect, tobacco smoke does not differ from other forms of smoke. However, the characteristic odour of tobacco smoke enables the source of irritation to be easily identifiable. Another difference is that the side-stream smoke of cigarettes, which is particularly rich in aldehydes, is often generated close to nose and eye level so that although the cumulative exposure to irritants present in it may be relatively low, there can be brief periods of heavy exposure to them.

IRRITANTS AND ASTHMA

Undoubtedly, exposure to airborne irritants affects atopic individuals adversely despite the fact that irritants such as nitrogen dioxide, sulphur dioxide and various aldehydes are not themselves allergens.

Increasing affluence, particularly in Europe and North America, but also elsewhere, has been associated with increasing prevalence of asthma, particularly among children and young adults, during the last 15-20 years. The present consensus view is that changes in housing and lifestyle underlie this increase. Mean indoor temperatures have been rising. Energy conservation has led to reducing ventilation. And these two factors combined with increased carpeting and soft furnishing have resulted in increased exposure, particularly to house-mite allergen, but also to allergens associated with infestation by cockroaches and with the keeping of animal pets (Platt-Mills, 1994; Cullinan and Newman Taylor, 1994).

An alternative theory that the incidence of childhood asthma has been rising because of increasing pollution outdoors by irritants such as nitrogen dioxide and sulphur dioxide present in vehicular exhaust fumes (Devalia *et al*, 1994) lacks credibility; firstly, because such irritants are not allergens, and secondly, because the prevalence of asthma has increased in areas of low air pollution as well as in big cities. On the other hand, the possibility that exposure to

irritant gases enhances the risk of sensitization by allergens is supported by data from several studies (eg. Soyseth *et al*, 1995).

Although irritant air pollutants do not increase the incidence of asthma as such, they do increase bronchial responsiveness in non-atopic as well as in atopic individuals (Forastiere *et al*, 1994). Insofar as the term 'asthma' is nowadays often rather loosely used to describe bronchial restriction in non-atopic individuals, the possibility must exist that some or all of the apparent increase in the incidence of asthma in recent years is in reality a consequence of changing standards in the use of diagnostic criteria plus a real increase in bronchial constriction attributable to increasing exposure to certain irritants.

The contribution of exposure to ETS to the risk of asthma in children has attracted much attention during recents years. On the one hand, the findings in many recent studies are consistent wih the conclusion that parental smoking in the home increases the risk of asthma. A suspicion that this is true remains after the influence of important confounding variables (eg. size of house, means of heating, size and ventilation of kitchen, family income, diet, keeping of pets, etc) are taken into account. On the other hand, the plausibility of this theory is questioned by the fact that during the period when the prevalence of childhood asthma has been rising, the prevalence of smoking has been falling.

Since the spectrum of products of incomplete combustion found in ETS is closely similar to that in smoke from other organic and/or fossil fuels, it makes sense to consider pollution from all these sources together. When Ostro *et al* (1994) did this, they found that indoor air pollution by the products of combustion, irrespective or whether they are derived from fireplaces, wood-stoves, gas stoves or tobacco smoking, exacerbate the respiratory symptoms in adult asthmatics.

ENVIRONMENTAL TOBACCO SMOKE (ETS)

Also, although since 1981 there has been a growing literature concerning the risk of lung cancer in non-smokers married to smokers compared with that in non-smokers married to non-smokers, there is no clear or unchallengable evidence that the former are at greater risk. The fact that smokers and ex-smokers who develop lung cancer are often misclassified as non-smokers, combined with the fact that smokers tend to marry smokers and non-smokers tend to marry non-smokers, is a source of bias in the interpretation of epidemiological studies (Lee, 1988). Over and above this, in such comparisons the need to collect data on important actual and potential confounding variables, including diet, occupation, and exposure to indoor air pollutants in the home, is rarely adequately met. In the case of diet, it is clear that non-smokers married to smokers commonly consume diets which are less cancer-protective than non-smokers married to non-smokers (Lee, 1992). Publication bias is also a problem in this area (Lee, 1992). Finally, though probably not an important source of bias, it is a matter of serious concern that the diagnosis

of lung cancer as the underlying cause of death is extremely unreliable unless an autopsy is carried out (Lee, 1994; Szende *et al*, 1994).

LUNG CANCER CALORIC INTAKE AND DIET

Mice of many strains are genetically prone to develop adenomas and adenocarcinomas of the lung spontaneously (ie. in the absence of any exposure to airborne irritants or carcinogens). Exposure to carcinogens, whether by inhalation, by the oral route or by parenteral injection, can increase the incidence of such tumours and predispose to their appearance earlier in life. However, a simple reduction in caloric intake (eg. to 75% of that consumed by mice fed *ad libitum*, reduces the incidence of spontaneously arising lung tumours dramatically (Conybeare. 1980). Rats are less prone than mice to develop adenomatous tumours of the lung spontaneously. However, we recently reported a significantly lower incidence of such tumours in calorie-restricted rats (80% of *ad libitum*) than in *ad libitum*-fed rats (Roe *et al*, 1995).

These observations, combined with evidence that calorie restriction leads to reduced cell proliferation in various tissues (Lok *et al*, 1990) are consistent with the theory that one determinant of lung cancer incidence in rats and mice is the rate of cell turnover.

Whether or not calorie-restriction influences the incidence of lung cancer in humans has not been investigated. However, there is considerable evidence that dietary factors generally influence lung cancer risk in non-smokers. Thus, Block *et al* (1992) reported that in all but 2 of 32 studies, after standardising for smoking habits, a low consumption of fruit and vegetable was associated with a 2.2 higher risk of developing lung cancer. The most plausible explanation of this finding is that antioxidants such as carotene and ascorbic acid present in fruit and vegetables protect the lung against DNA damage due to oxidants produced endogenously during the metabolism of food and those liberated by macrophages involved in inflammatory processes (Roe, 1994). These findings and theories are clearly relevant to the assessment of lung cancer risk resulting from exposure to irritants and have been expertly reviewed by Cross *et al* (1994).

CONCLUSION

1. The relationship between irritation and disease of the respiratory tract is not straightforward.

2. The anatomy of the respiratory tract is complex.

3. The term "irritation" has different meanings.

4. A wide range of factors, both chemicals and microbial, and either singly or in combination, can act as irritants.

5. It has only recently been accepted that non-genotoxic mechanisms can cause cancer and that mutagens are produced endogenously.

6. Research based on the use of laboratory animal models have sometimes been over-simplistic.

7. More attention needs to be paid to the influence of dietary factors in relation to lung cancer risk.

8. Increasingly affluent human lifestyle in Western countries is favouring the prevalence of house-mite infestation and the development of asthma.

9. Irritants trigger asthma attacks but are not themselves sensitizing.

10. Cooking and heating fumes are serious contributors to indoor air pollution in poor countries.

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