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Damage to the nasopharyngeal mucosa induced by current levels of urban air pollution: a field study in lambs

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Damage to the nasopharyngeal mucosa induced by current levels of urban air pollution: a field study in lambs. M. Gulisano, S. Marceddu, A. Barbaro, A. Pacini, E. Buiatti, A. Martini, P. Pacini. ©ERS Journals Ltd 1997.

ABSTRACT: This study concerns the effects of urban air pollution on the nasopharyngeal epithelium, with the aim of evaluating the possible harmful activity of levels of atmospheric pollution which are not currently considered to be dangerous.

Over a 3 month period, 10 lambs kept in a zone characterized by numerous vehicles were sacrificed at regular intervals, and their nasopharyngeal mucosa was examined by scanning electron microscopy and image analysis. Two lambs kept in a rural area were used as controls. The local levels of some airborne contaminants (NO_x, NO_2, NO, SO_2, CO and particulate matter with aerodynamic diameter ≤10 µm (PM10)) were monitored throughout the experiment.

The urban air had an irritating effect, inducing hypersecretion of mucus and morphological damage to the ciliated epithelium. These alterations increased with the duration of exposure to urban air and with increasing pollution levels, although the levels remained below current legal levels.

We conclude that the harmful effects of airborne contaminants are probably underestimated. Moreover, physicochemical evaluation of pollution parameters should be complemented by morphological study of upper respiratory epithelium in exposed animals, since this mucosa is a sensitive target for irritating agents.
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The mucosa of the upper respiratory tract (URT) is exposed to the first impact of almost all the airborne irritating agents [1]. This mucosa consists of cells that almost immediately undergo morphological changes the complex alterations induced at subcellular level by the action of these agents [2]. Therefore, the morphological pattern of this mucosa is greatly altered by the action of airborne pollutants, which are at present increasing [1, 3].

A general reduction of atmospheric pollution cannot be expected in the near future, despite the World Health Organization (WHO) statement in 1985 "by 1995 all the people of the European region should be effectively protected against recognized health risks from air pollution".

Air pollution can vary greatly, depending both on its source (traffic, factories, power stations, *etc.*) and on the geographical, climatic and environmental characteristics of the area under consideration [4]. Nevertheless, in most large cities, vehicles are a very substantial source of pollution [4], spreading many inhalable agents into the atmosphere, such as gas (NO_2, SO_2, CO, O_3, benzene, *etc.*), smoke, dust, heavy metal particles (lead, cadmium, *etc.*), and other substances. Such agents can reach irritating levels, causing both short- and long-term damage to the respiratory mucosa, as was demonstrated both in animals (rabbits) [1], and humans [5].

In humans, the morphological signs of short-term irritating activity consist firstly of a hypersecretory reaction of the mucous glands and goblet cells, whilst, at the same time, cilia rapidly decrease in size and number [6].

As a result of long-term exposure, loss of the normal pseudostratified pattern of the epithelium can occur, with cuboidal and then squamous metaplasia [2, 7, 8]. Mucus tends to decrease and to change its physicochemical quality, as was demonstrated in humans [5, 6] and in rats [9], both for environmental [5] and occupational [9] exposures.

In Italy, the Public Health Authorities decided upon "attention levels" (NO_2 200 $\mu g \cdot m^{-3}$, SO_2 125 $\mu g \cdot m^{-3}$, CO 15 $ug \cdot m^{-3}$, O_3 180 $mg \cdot m^{-3}$, and particulate matter 150 $\mu g \cdot m^{-3}$) and "alarm levels" (double the attention levels) for these substances, and adopted preventive measures (such as prohibition of vehicles) when air pollutant levels exceeded these limits. Nevertheless, the actual irritating activity of urban air was demonstrated in experimental animals (rabbits) [1].

In view of this, it was considered of interest to look for a possible correlation between the appearance of morphological alterations in the epithelium of the nasopharynx and the levels of air pollution in a modern city, even in the absence of exceptionally serious pollution episodes. Such a correlation would indicate both that air pollution induces gradable and quantifiable damage, and that morphological evaluation is a suitable instrument for monitoring pollution.

Material and methods

The study was carried out on 12 lambs born on a breeding-farm far away from polluting sources (factories, traffic, *etc.*). Ten days after birth, 2 animals were left in the country as controls, while 10 were taken to Florence, and kept in a zone with a high traffic level. The initial purpose of the experiment was to evaluate the energy value of zootechnic fodder. No tissues other than the nasopharyngeal mucosa were available for evaluation.

During the whole experiment (from April to June 1994), the local daily mean level of the following substances was obtained from an air pollution monitoring station: total nitrogen oxides (NO_x), nitric oxide (NO), nitrogen dioxide (NO_2), carbon monoxide (CO), sulphur dioxide (SO_2), and particulate matter with aerodynamic diameter <10 µm (PM10).

The animals were sacrificed individually at intervals of 9 days, from April 9 to June 29, 1994. The experiment was performed according to the Helsinki convention for the use of animals. According to Italian law, the lambs were sacrificed by means of a sticking knife, after captive bolt stunning. The two control animals were sacrificed after 50 and 100 days.

Morphological evaluation

From each sacrificed animal, fragments from the posterosuperior region of the nasopharynx were obtained and processed, following current procedures [1, 6], for scanning electron microscopic (SEM) study. The observations were carried out using a Zeiss DSM 962 microscope.

For each animal, at least 10 areas of 2,500 µm² (50 × 50 µm), randomly chosen along the entire surface of the posterosuperior zone of the nasopharynx, were evaluated. Each area was accurately observed over the entire surface, without knowledge of the origin of the sample.

Quantitative analysis

With the aim of giving some quantitative assessment to this study, the 50 × 50 µm areas observed were examined by means of an image analyser (Quantimet 600 S; Leica, Cambrigde, UK). For each area, the following parameters were evaluated: 1) percentage of the total surface lined by cells equipped with microvilli and devoid of cilia; 2) percentage of the total surface covered by mucus; 3) percentage of the total surface lined with evident goblet cells; and 4) percentage of the total area lined with necrotic or severely damaged epithelium.

Results

Throughout the period of study, the levels of the pollutants did not exceed the legal air quality standards, even in those periods in which relatively high levels were reached, such as April 17–24, May 8–19, or June 12–29 (fig. 1).

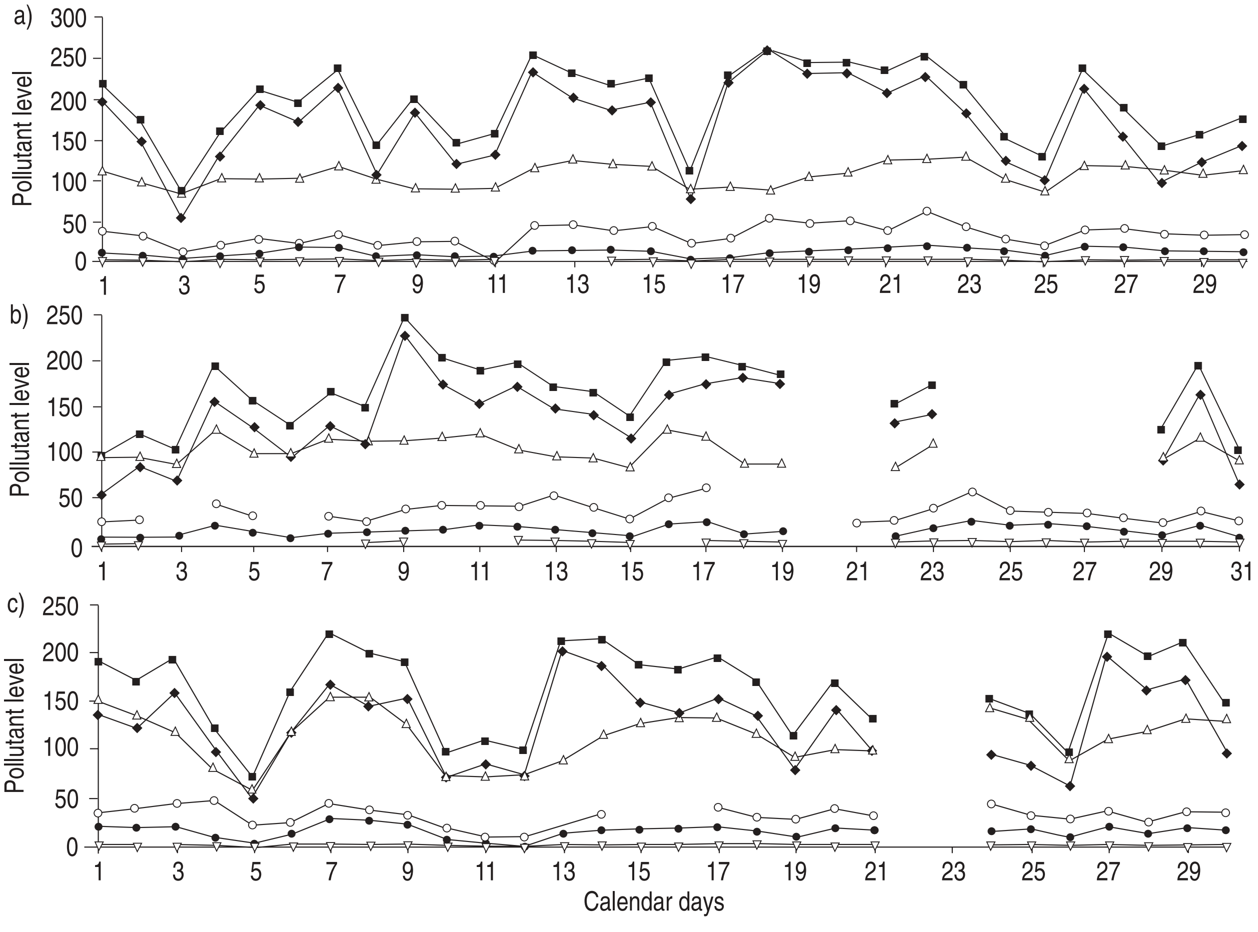

Fig. 1. – Levels of pollutants in the period: a) April 1994; b) May 1994; and c) June 1994. —●—: sulphur dioxide ($\mu g \cdot m^{-3}$); —■—: total nitric oxides of nitrogen (ppb); —◆—: nitric oxide ($\mu g \cdot m^{-3}$); —△—: nitrogen dioxide ($\mu g \cdot m^{-3}$); —▽—: carbon dioxide ($mg \cdot m^{-3}$); —○—: particulate matter with aerodynamic diameter ≤10 mm (PM10) ($\mu g \cdot m^{-3}$)

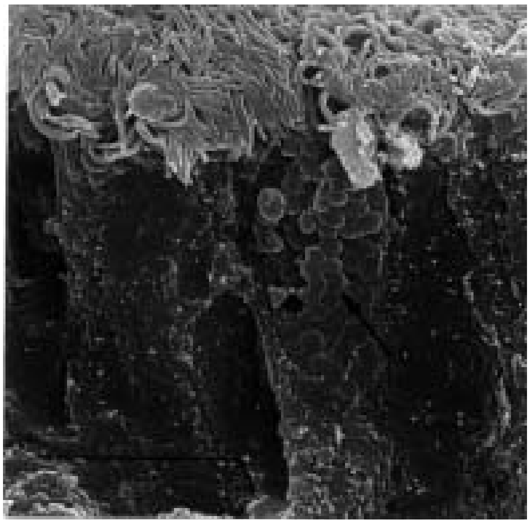

Fig. 2. – Control. Lateral view of nasopharyngeal epithelium. Note the globular masses of mucus in a goblet cell (arrow). (Internal scale bar=10 μm).

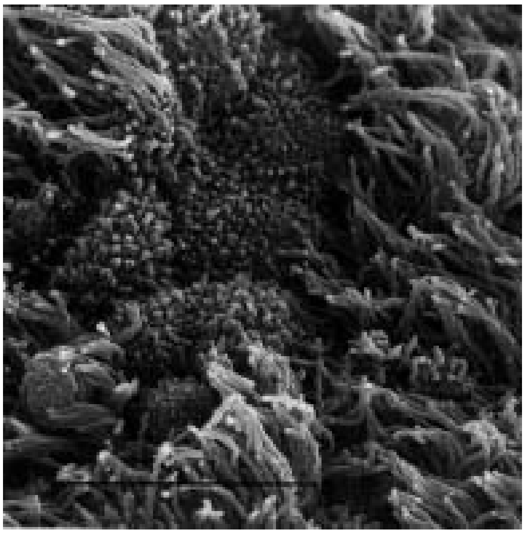

Fig. 3. – Control. Some microvilli-bearing cells are to be seen amongst the ciliated cells. (Internal scale bar=10 μm).

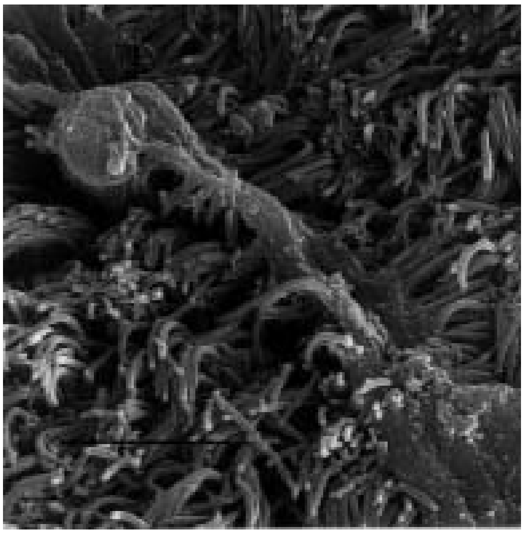

Fig. 4. – Animal sacrificed on April 9, 1994. Abundant mucus should be noted, coagulated in a long filament (Internal scale bar=5 μm).

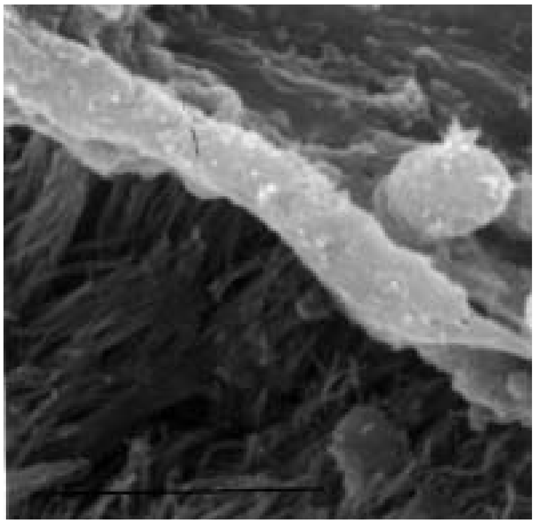

Fig. 5. – Animal sacrificed on April 18, 1994. A small globular mass flows into the mucous blanket covering the ciliated cells (Internal scale bar=10 μm).

The nasopharyngeal mucosa of the two control animals was lined by a pseudostratified epithelium, with ciliated cells and interspersed goblet cells (fig. 2). Isolated, irregularly polygonal cells, had slightly dome-shaped apices equipped with microvilli (fig. 3).

In the exposed animals, it was already possible to observe an increased presence of mucus after 9 days. The mucus was coagulated in filaments or flakes (fig. 4), or constituted a blanket, into which small globular masses flowed, tenaciously adhering to the cellular apices (fig. 5). As demonstrated in figure 6, the mucus eventually covered more than 13% of the epithelial surface at that time. Nevertheless, after May 6, this trend seemed to be reversed, and the mucus showed a decrease.

The goblet cells were numerous and showed signs of intense activity (fig. 7). Until June 11, the goblet cells seemed to increasingly active and numerous; after this date, their number stopped increasing.

In the animals sacrificed in late April, it was possible to observe numerous bacteria adhering to the cilia

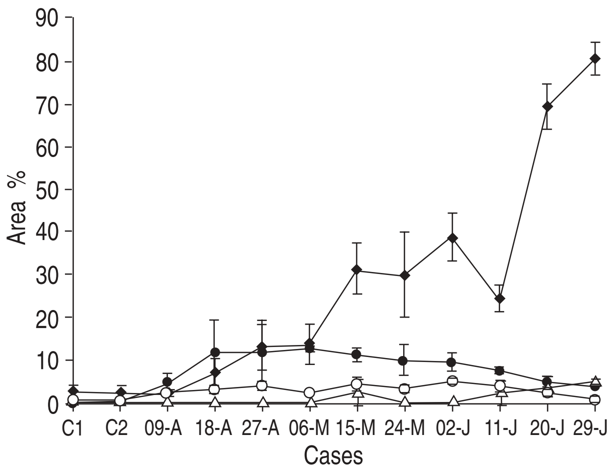

Fig. 6. – Percentage of the total cell surface covered by: nonciliated cells (◆); goblet cells (○); mucus (●); and necrotic epithelium (△). The x-axis indicates the cases, identified by C (control) or by the date of the sacrifice (A=April, M=May, J=June). The y-axis indicates the area, expressed as the mean value of the percentages measured in all of the 10 fields evaluated. Values are presented as mean±SD.

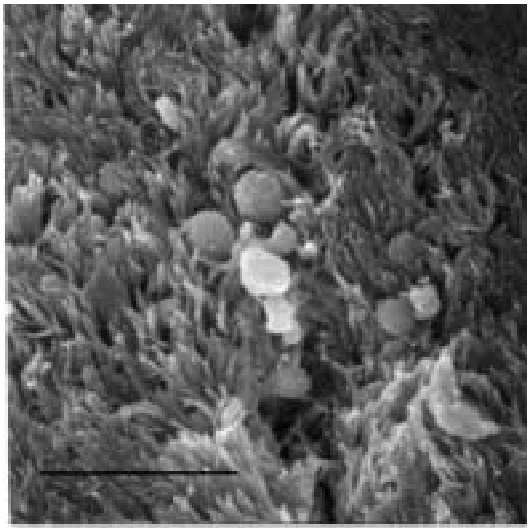

Fig. 7. – Animal sacrificed on June 2, 1994. Numerous and active goblet cells are evident. (Internal scale bar=20 μm).

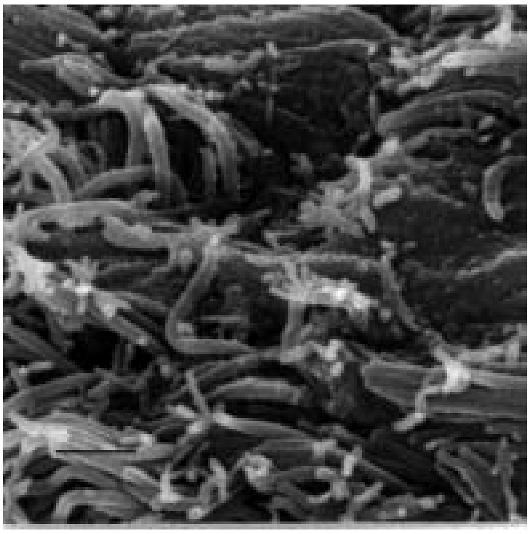

Fig. 8. – Animal sacrificed on April 27, 1994. Numerous bacteria are evident, adhering to the cilia. (Internal scale bar =1 μm).

Fig. 9. – Animal sacrificed on April 27, 1994. A degenerated ciliated cell is detaching itself from the epithelial surface. (Internal scale bar =5 μm).

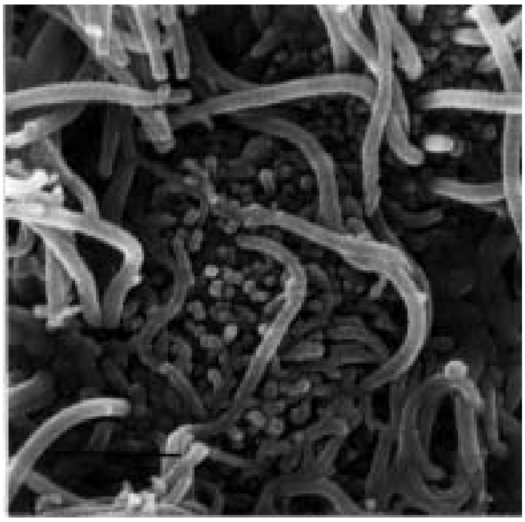

Fig. 10. – Animal sacrificed on May 24, 1994. On a cellular apex, microvilli are evident as a consequence of the disappearance of the cilia. (Internal scale bar =2 μm).

Fig. 11. – Animal sacrificed on June 11, 1994. In numerous and wide areas, the microvilli-bearing cells have almost completely replaced the ciliated cells. (Internal scale bar =20 μm).

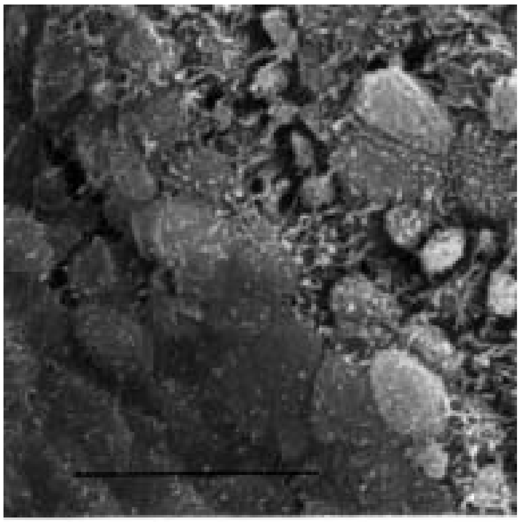

Fig. 12. – Animal sacrificed on June 29, 1994. A wide area lined with damaged cells is evident. Note the smooth, bloated apices, and the poorly defined cellular boundaries. (Internal scale bar =20 μm).

(fig. 8). At the same time, from April 27, ciliated cells showed progressive signs of damage, and degenerated ciliated cells detaching themselves from the epithelial surface were to be seen (fig. 9). Moreover, the cilia often decreased in number and size, thus exposing the underlying microvilli (fig. 10).

The cells equipped with only apical microvilli became increasingly numerous (fig. 2), until they almost completely replaced the ciliated cells, becoming the major surface elements (fig. 11).

In the animals sacrificed during the period from June 11 to June 29, corresponding to an almost constantly increased concentration of contaminants (fig. 1), and to the end of the study period, areas lined with apparently acutely damaged cells, showing bloated apices and poorly defined boundaries commonly occurred (fig. 12); this is also demonstrated in figure 2.

Discussion

In the present study, only the nasopharynx was considered, since it appeared more suitable than other areas of the airways to evaluate the action of airborne irritants [10]. In the ovine nasal cavities there are normally large areas of squamous epithelium, in which it is very difficult to demonstrate the morphological expression of irritative changes. On the other hand, the tracheal and bronchial epithelia show other variables, such as the presence of Clara cells, which could complicate the evaluation. It is true that in the nasopharynx different types of epithelia (pseudostratified, squamous, lymphoid, transitional) can coexist, but the posterosuperior area generally shows a more constant presence of pseudostratified epithelium [10]. This was demonstrated by the images obtained from the control animals in the present study, even if there were, admittedly, a very low number of them.

The results seem to confirm previous findings [1, 11], demonstrating that air pollution effectively acts as an irritating factor on the URT mucosa. Moreover, the increased bacterial presence in the damaged epithelium should be noted, since there is consistent evidence for increased infective risks in subjects exposed to polluted atmosphere [4, 12]. Concerning the causes of this presence, one could envisage that the alterations of the mucociliary clearance facilitate bacterial proliferation.

The appearance of respiratory epithelium differed remarkably between the control animals kept in a rural area and those kept in the city. The most sensitive indicators of epithelial suffering are secretion of mucus and loss of cilia [6, 13–15]. It is impossible to determine whether the goblet cells actually increased in number or were simply more visible, due to an increased activity [6, 14]. It should be noted that proliferation of mucous cell and hypersecretion of airway mucus are generally considered to be notable consequences of the irritating activity of some contaminants [13]. It is also noteworthy that the goblet cell number tended to decrease after June 11; previous research [6] has demonstrated a similar pattern in chronic nasal inflammation.

Another interesting point is that pollution peaks correspond to more critical conditions in epithelial appearance. In the peaks located in the early period of the experiment, damage to and degeneration of the ciliated cells can be noted. The ciliated cells appear to be the most sensitive elements [15–18], and surely the cells in which damage is most evident from a morphological standpoint. Following these observations, it is possible to propose these cells as indicators of the damage due to air pollutants [4, 6, 19]. In the later period of the experiment, pollution peaks corresponded to the presence of areas lined with necrotic or severely damaged cells. It is reasonable to suppose that damage was more serious in the later period, both because air conditions were worse, and because the pollutants were acting on an epithelium that had already suffered the effects of prolonged exposure to city pollution.

Nevertheless, it should be noted that severe morphological damage was evident even though pollutant concentrations never reached the alarm level during the period considered. This could mean that there was another airborne agent that was not measured, but which did reach dangerous levels. This hypothesis should be verified considering other possible pollutants. However, it is very difficult to identify all possible air contaminants, since for air (unlike, for example, drinking-water) there is no centrally supplied and controlled source [4].

Alternatively, one might hypothesize that levels considered as innocuous can in fact induce acute cellular suffering. This hypothesis seems to disagree with current reports [4]. On the other hand, SCHWARTZ [20] demonstrated a significant increase in mortality due to respiratory disease correlated to a small increase of particulate matter, considered as a marker of air pollution. Moreover, more consistent information on a combined action of air pollutant agents is needed [21–23], since the combined (additive or synergistic) toxicity might well account for effect levels being lower than expected.

It should also be remembered that June 1994 was a month of constant, intense sunshine. It is well-known that the sun's rays cause an increase of photochemical compounds in polluted atmosphere [4]. However, the level of NO_2, which can be assumed to be a marker of photochemical pollution [4], never exceeded the attention level of 200 $\mu g \cdot m^{-3}$. Nonetheless, photochemical action able to modify the composition or action of this pollutant "cocktail" cannot be ruled out, and deserves specific examination from a chemical standpoint [22].

Certainly, the low number of animals employed does not endow these conclusions with a high statistical value. Therefore, in consideration of the relevance of the results, it would be interesting to transfer this model to a larger population, and possibly to human volunteers, who should undergo nasopharyngeal biopsy. Nevertheless, it should be emphasized that more collaboration and increased interest on the part of the Health Authorities in the problem of research into atmospheric pollution is desirable.

Finally, it is reasonable to assert that the morphological evaluation of the URT epithelium is a highly sensitive technique for highlighting the harmful activity of air pollutants [11, 15], which is not identified simply by chemical survey. The originality of the present study lies, in our opinion, in the "field approach", which made it possible to evaluate the real action of the pollutant cocktail, whose composition cannot be completely analysed

[4]. Computerized image analysis could not make up for the mere descriptive character of such evaluation, whilst the observation of morphobiological effects could integrate the significance of chemical/physical survey methods. If possible, adaptive phenomena able to modify the behaviour of the upper respiratory tract epithelium in animals permanently living in cities should be evaluated by long-term studies.

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