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Effects of Air Pollution on Passerine Birds and Small Mammals

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Abstract. The effects produced by emissions from coal-fired power plants, including mainly SO_2, NO_x and particulates, on natural populations and caged specimens of birds and small mammals were studied. The field-captured species used to evaluate these effects were passerine birds: *Parus major* (coal tit) and *Emberiza cia* (rock bunting), and the rodent *Apodemus sylvaticus* (wood mouse). In parallel to this study on animals captured in the field, we used other animals, *Mus musculus* (house mouse) and *Carduelis carduelis* (goldfinch) which were placed in cages near the source of pollution. Some of the animals were killed and their tracheas were removed and prepared for conventional optic studies (1000×) and electron microscopy (TEM and SEM). The results show that atmospheric air pollutants from coal-fired power plants produce alterations in the tracheal epithelium. In passerine birds, an increase in the mucus which covers the tracheal epithelium, shortening of the cilia, and increase in the number of secretory granules and vesicles were observed. In mammals, variation of the uniformity of the pseudostratified epithelium with a wide stratum of mucus, shortening of the cilia, and increase in the number of secretory granules were observed.

There are very few studies of the effects of atmospheric pollutants on natural populations of wild animals. We have located only two references that relate to field studies (Wolak 1979) and those realized in Montana (1975).

In contrast, there are many laboratory studies. The references are extensive and working conditions are highly controlled and constant (temperature, humidity, pressure, inhaled gases, water, food, and size of the chamber where animals are placed). It was thus decided to perform a field study, taking into consideration that gaseous emissions are diluted in the atmosphere before they are inhaled by animals. Moreover, the effects produced by gases alone or in combination are surely very different (Schlesinger 1987). Extrapolation of laboratory studies involving single pollutants to complex natural situations is difficult.

Another important factor is the animals used. In laboratory studies, animals are specifically chosen and experiments are performed in controlled conditions, but results are not necessarily applicable to other wild species.

The interest in studying and following up the effects in wild conditions starts from the intervention of factors which are usually not evaluated, such as temperature, humidity, wind speed, rainfall, and hours of sunshine. Many field studies evaluate the impact of the atmosphere on lichens or vegetation in general. We have included species whose physiology is more similar to that of man than to lichens. Among the wild mammals we chose *Apodemus sylvaticus* (wood mouse) and among the wild passerine birds, *Emberiza cia* (rock bunting) and *Parus major* (coal tit). In addition, we chose other animals, which were placed in a field, housed in protected cages, at about 2 km from the source of emissions in order to study the long-term effects in a more controlled way. As mammal we used *Mus musculus* (house mouse) and as the passerine bird, *Carduelis carduelis* (goldfinch) (Table 1).

These studies were carried out in the area of Cercs (Bergueda, Catalonia, northeast Spain) in which there is a coal-fired power plant which releases, among other pollutants, SO_2 and NO_x, which are measured every thirty minutes at the plant, and in St. Jaume de Frontanya and St. Llorenç dels Morunys, which are unpolluted areas. These areas are very similar to Cercs in altitude, temperature and vegetation. On the other hand, the relief in Cercs allows gases from the plant to rise up a cliff and into the high plateau (Vallcebre), where they condense. Conditions in this calcareous massif of characteristic relief and the continuous monitoring of the composition of the atmosphere (Table 2) allowed us to measure the environmental conditions to which the various vertebrate communities are subjected.

Although the studies deal with the effects of air pollution on lung, kidney and liver, hematologic parameters: hemoglobin-hematocrit values, number of erythrocytes, number of leukocytes, VCM (mean corpuscular volume), HbCM (mean corpuscular hemoglobin), CHbCM (mean corpuscular hemoglobin concentration) and fluctuations in animal communities, our objective in this paper was to evaluate the effects of atmospheric pollutants from coal-fired power plants on the tracheal epithelium of passerine birds and small mammals.

Materials and Methods

Apodemus sylvaticus were captured, using non-lethal wooden mouse traps. *Emberiza cia* and *Parus major* were captured in Japanese meshes. *Carduelis carduelis* were captured in the wild and *Mus musculus* from our laboratory were placed in protected cages near the emissions and kept there for six months (January–June 1991). Animals

Table 1. Species location of capture or cage, and number of animals exposed at each site

Species	Location	Number of specimens	Polluted area
Wild caught			
P. major	Cercs	2	+
P. major	S. Jaume/S. Llorenç	2	−
E. cia	Cercs	2	+
E. cia	S. Jaume/S. Llorenç	3	−
A. sylvaticus	Cercs	3	+
A. sylvaticus	S. Jaume/S. Llorenç	2	−
Caged			
C. carduelis	Cercs	3	+
C. carduelis	S. Jaume/S. Llorenç	4	−
M. musculus	Cercs	2	+
M. musculus	S. Jaume/S. Llorenç	2	−

Table 2. SO_2 and NO_x values (μg/Nm3) measured during January and March–June 1991 at St. Corneli's Monitoring Station (Cercs)[a]

Month	January	March	April	May	June
Mean value for SO_2	17.	25.	384.	619.	15.
Maximum value for SO_2	659.	1114.	2500.	2162.	255.
Mean value for NO_x	25.	10.	11.	16.	5.
Maximum value for NO_x	1172.	344.	356.	1221.	225.

[a] For February no data was available.

from all groups were killed, and tissue specimens were prepared as described below.

Tissue specimens were prepared for conventional electron microscopy. Tracheas were removed, fixed with 2% formaldehyde-2.5% glutaraldehyde PBS buffer solution and post-fixed in 1% osmium tetroxide-0.1 phosphate buffer solution before acetone dehydration and Spurr (mixture of several resins) embedding. Ultrathin sections were mounted on conventional grids and observed at transmission electron microscope (TEM) (P-200). Semithin sections were also obtained, which were stained with methylene blue solution before observation at light microscopy (1000×). Sections were also prepared for conventional studies on a scanning electron microscope (H-2300).

For the detection of mucopolysaccharides and glycoproteins, some semithin sections were stained and processed according to the procedure of PAS (periodic acid Schiff). The parameters used to evaluate the section were: intercellular distance, percentage of ciliated cells, mean length of cilia, presence/absence of mucus, and presence and size of secretory granules and glycoprotein-mucopolysaccharide vesicles. In mammalian sections, we also measured the packing and distribution of the nuclear chromatin as well as height and thickness of the pseudostratified tracheal epithelium.

Statistical analysis: The Chi-square test was used to test the significance of differences found.

Results

The species location of capture or cage, and the number of animals exposed at each site are summarized in Table 1. The atmospheric emissions of SO_2 and NO_x, supplied by FECSA, the electricity company which monitors gases and particles at the Cercs site are listed in Table 2.

Comparison of the tracheal epithelia obtained by light microscopy (1000×) and electron microscopy (TEM and SEM), showed the following.

Passerine Birds

Carduelis carduelis (Figures 1, 2, and 3)

The total length of the tracheal epithelium used to measure the parameters was 1,679 μm in control animals and 1,471 μm in animals from Cercs.

The three caged specimens from the polluted area (Cercs) had a uniform mucus layer in the tracheal lumen covering the cilia. These cilia had an average length of 1.8 μm above the mucus. Specimens from the non-polluted areas did not show mucus, and cilia were much longer (4.3 μm). This difference could be due to the presence of mucus, which makes it difficult to measure cilia length.

We also observed in control animals that the intercellular distance on the tracheal surface was about 7 μm on average, whereas in the species from polluted areas it was 6 μm. This could be due to the fact that 71% of the cells were ciliated in control species and 29% of the cells were non-ciliated cells, while 59% of the cells were ciliated and 41% were non-ciliated in the animals from Cercs. In animals from polluted area there was an increase of the non-ciliated cells versus ciliated cells.

Inside the secretory cells were distinguished secretory granules (dark tone) and glycoprotein-polysaccharide vesicles (light tone), the distribution and size of which varied according to the polluted or unpolluted zones. In sections of control animals the vesicles were rare and the few we observed were less than 1 μm in length. In most of the animals from polluted areas, there were about 33% more vesicles and their size was between 1 and 3 μm.

The secretory granules in cells from control animals were joined together (60% had a diameter of over 0.3 μm) and they were near to the tracheal lumen and only slightly dispersed in the cytoplasm. In samples of animals from polluted area the pine formed by the secretory granules was smaller (40% had a diameter of over 3 μm) but the number of dispersed granules was much higher (Table 3).

Emberiza cia (Figure 4)

The total length of the tracheal epithelium used to measure the parameters was 990 μm in field-trapped control birds and 1,470 μm in birds from Cercs.

The length of the cilia which move the air in the tracheal lumen was 2.3 μm in animals from polluted area and 3.1 μm in control animals. The cilia moved freely, because there was no mucus, but the number of ciliated cells varied: 71% in control tissue and 65% in tissue from Cercs animals.

As in *Carduelis carduelis*, in sections of control animals we observed that secretory granules formed a pine and in 65% of cases they were more than 3 μm in diameter, and there were very few dispersed granules.

In animals from Cercs, this association of secretory granules was only above 3 μm in diameter in 30% of cases, but many were dispersed in the cytoplasm. 80% of the vesicles of secretory cells measured between 1 and 3 μm, compared with 30% in control (Table 3).

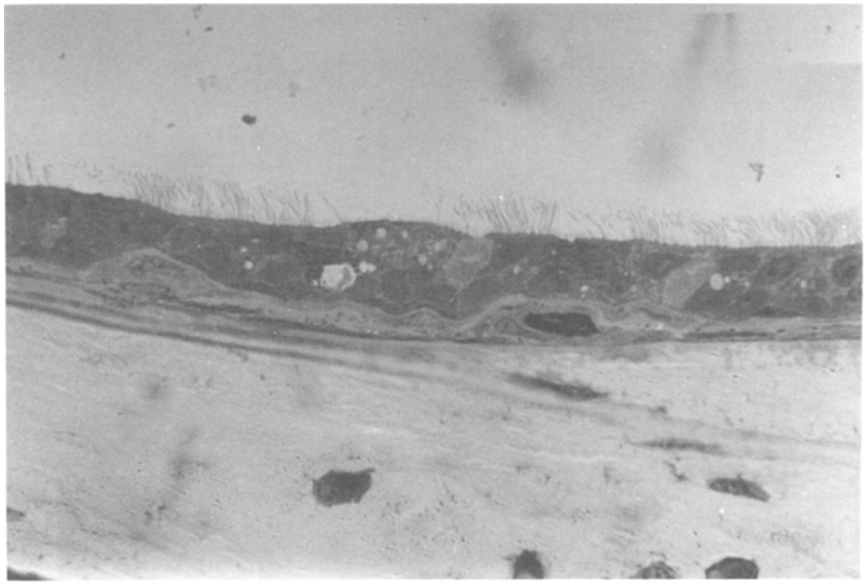

Fig. 1. Transverse tracheal section of *C. carduelis* from control zone. There is no mucus. Apical distribution of secretory granules. (Light microscope 1000×)

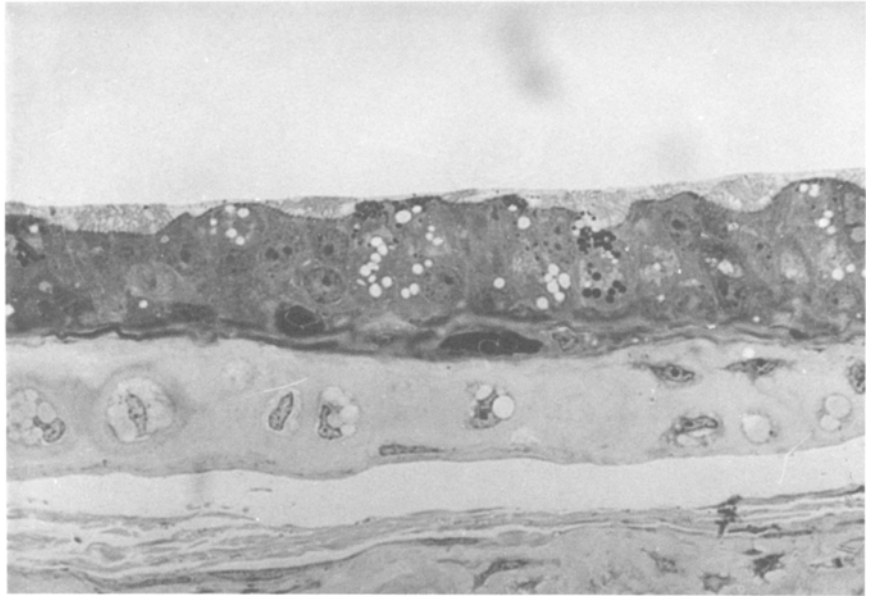

Fig. 2. Transverse tracheal section of *C. carduelis* from polluted zone. Great quantity of mucus and glycoprotein-mucopolysaccharide vesicles which are quite large and dispersed in the cytoplasm. (Light microscope 1000×)

Parus major

The total length of the tracheal epithelium used to measure the parameters was 355 μm in field-trapped control birds and 601 μm in birds from Cercs.

The differences between animals from polluted and non-polluted areas were null when sections were observed by light microscopy. However, a slight stratum of mucus was distinguished over the tracheal epithelium when observed on the electron microscope (TEM). The parameters analyzed were the same:

The mean intercellular distance was 5.2 μm
60% of the cells were ciliated
There were very few vesicles, and they were distributed in the cytoplasm
There were very few secretory granules
Absence of mucus in control animals, but a slight layer of mucus in animals from polluted area was observed

Small Mammals

Apodemus sylvaticus (Figures 5 and 6)

The total length of the tracheal epithelium used to measure the parameters was 998 μm in field-trapped control animals and 1,217 μm in animals from Cercs.

Animals from Cercs area had a stratum of mucus on their tracheal epithelium, with a thickness of about 2.9 μm. The mean length of the cilia was 2.3 μm whereas that in animals from the non-polluted areas was about 3 μm. The layer of mucus sometimes made measurement difficult.

In animals from the Cercs area, the mean intercellular distance was 5.9 μm and the percentage of ciliated cells was 52%,

Fig. 3. Transverse tracheal section of *C. carduelis* from polluted area. Tracheal lumen with mucus and particles. (Transmission electron microscope 11.200×)

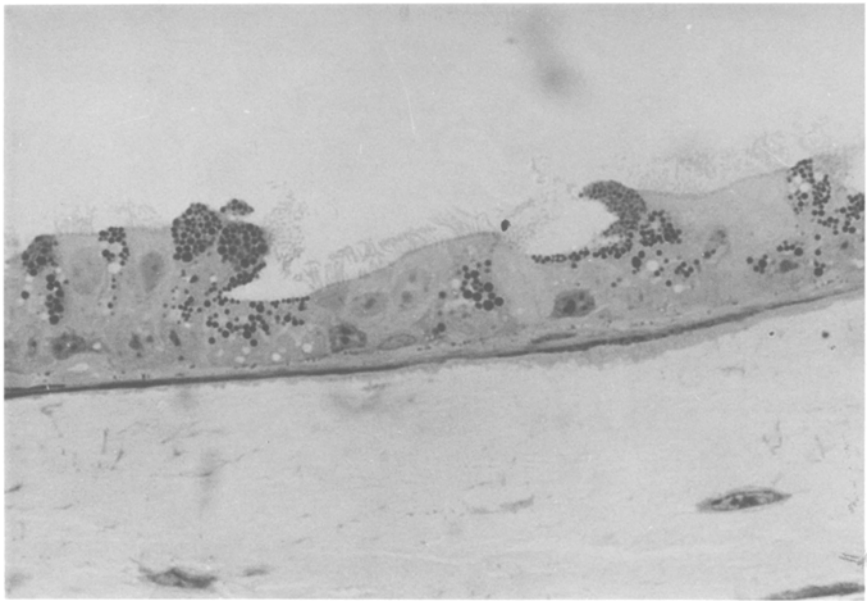

Fig. 4. Transverse tracheal section of *E. cia* from polluted area. Secretory granules with top distribution are also dispersed in the cytoplasm. Glycoprotein-mucopolysaccharide vesicles are observed. (Light microscope 1000×)

whereas results for control animals were 6.5 μm and 33%, respectively. We could not measure the percentage of ciliated cells in one of the animals (Cercs area) because of a severe graze on the tracheal epithelium.

Pseudostratified tracheal epithelium thickness was around 15.2 μm in animals from the polluted area. In control animals this thickness was 12.3 μm. A large number of secretory granules and mucopolysaccharide-glycoprotein vesicles were observed in animals from polluted areas, but these phenomena were absent in control animals (Table 4).

According to the distribution, quantity, and packing of nuclear chromatin we have differentiated three types of nuclei, which probably correspond to stages of synthesis:

Type a—Scarce chromatin with peripheric distribution
Type b—Chromatin distributed in one or two peripheric clusters

Table 3. Measures of several cellular parameters on *Carduelis carduelis* and *Emberiza cia* from polluted and non-polluted areas

	Results on *C. carduelis*		Results on *E. cia*	
	Control area	Polluted area	Control area	Polluted area
Mean intercellular distance	7 μm	6 μm	6 μm	6 μm
% Ciliated cells	71%	59%	71%	65%
Size of glycoprotein-mucopolysaccharide vesicles	80% 1 μm	70% 1–3 μm	30% 1–3 μm	80% 1–3 μm
Presence of mucus	−	+	−	−
Ciliar length	4.3 μm	1.8 μm	3.1 μm	2.3 μm

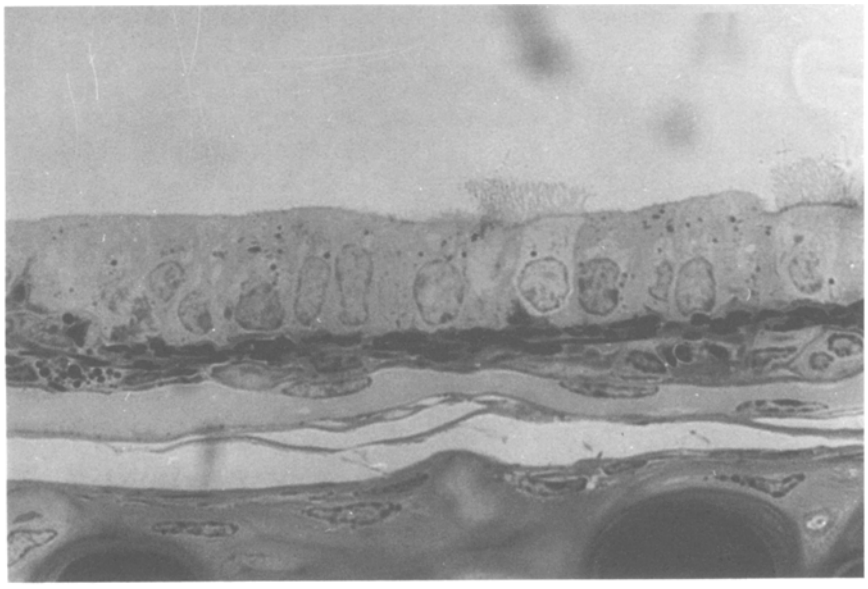

Fig. 5. Transverse tracheal section of *A. sylvaticus* from control zone. Few secretory granules and no mucus is observed. (Light microscope 1000×)

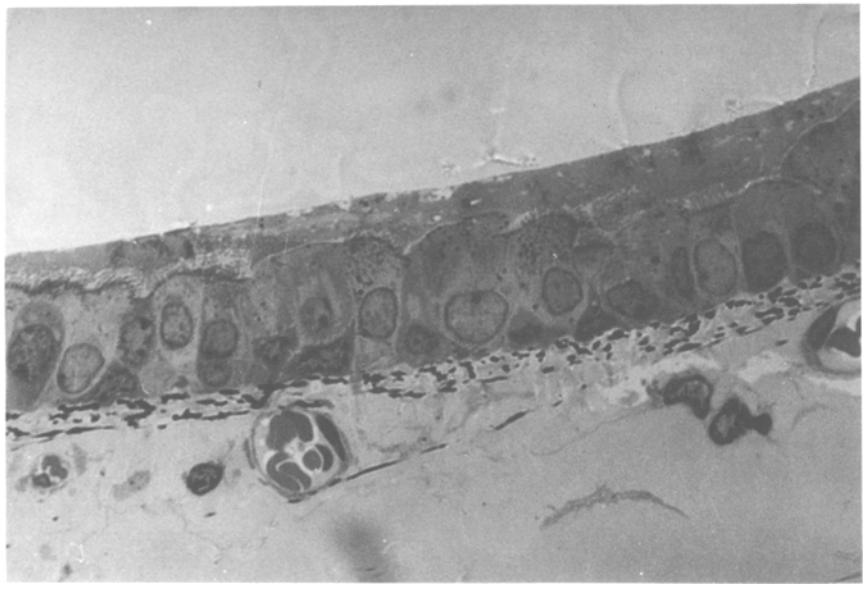

Fig. 6. Transverse tracheal section of *A. sylvaticus* from polluted area. There is a stratum of mucus covering the cilia and a large quantity of secretory granules. (Light microscope 1000×)

Type c—Large quantity of chromatin distributed in more than two clusters all around the nucleus

Mus musculus (Figures 7, 8, and 9)

The total length of the tracheal epithelium used to measure the parameters was 393 μm in control caged mice and 724 μm in mice held in cages at Cercs.

Some of the histologic differences between *Apodemus sylvaticus* captured in the control areas and polluted areas, such as the presence of mucus and lower ciliar length, were also observed in specimens of *Mus musculus* which were in captivity.

Animals from the polluted area had a layer of mucus with a thickness of 5.4 μm, but control animals had no mucus. This mucus also covered the cilia (2.7 μm mean length). In control animals, the mean ciliar length was 4.2 μm. In control animals,

Table 4. Measures of several cellular parameters on *Mus musculus* and *Apodemus sylvaticus* from polluted and non-polluted areas

	Results on *A. sylvaticus*		Results on *M. musculus*	
	Control area	Polluted area	Control area	Polluted area
Mean intercellular distance	6.5 μm	5.9 μm	6.3 μm	7.8 μm
% ciliated cells	33%	52%	29%	53%
Presence of mucus	−	+	−	+
Ciliar length	3.1 μm	2.3 μm	4.2 μm	2.7 μm
Chromatin a	25.5%	22%	12%	26%
Chromatin b	42.5%	61%	64%	67%
Chromatin c	33%	17%	24%	7%

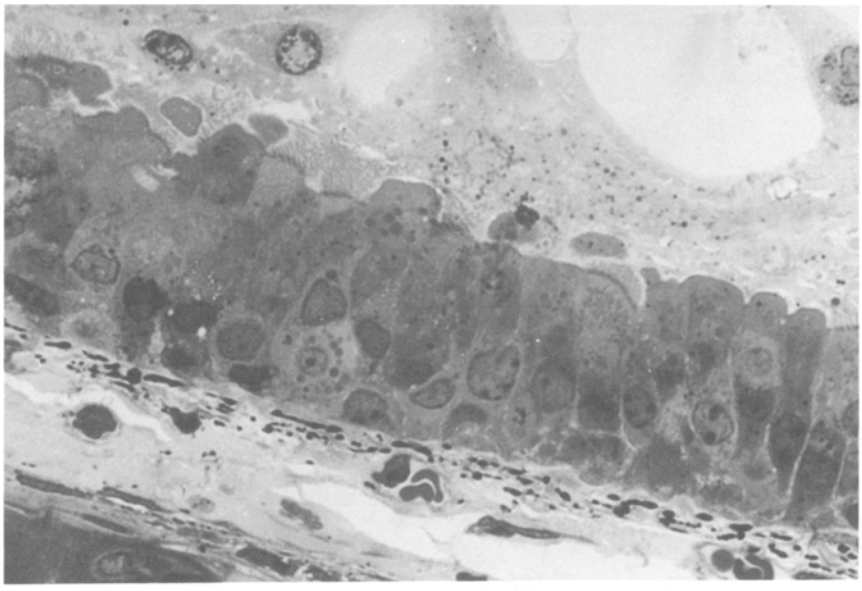

Fig. 7. Transverse tracheal section of *M. musculus* from polluted zone. Great quantity of mucus and secretory granules are observed. Cellular disorder. (Light microscope 1000×)

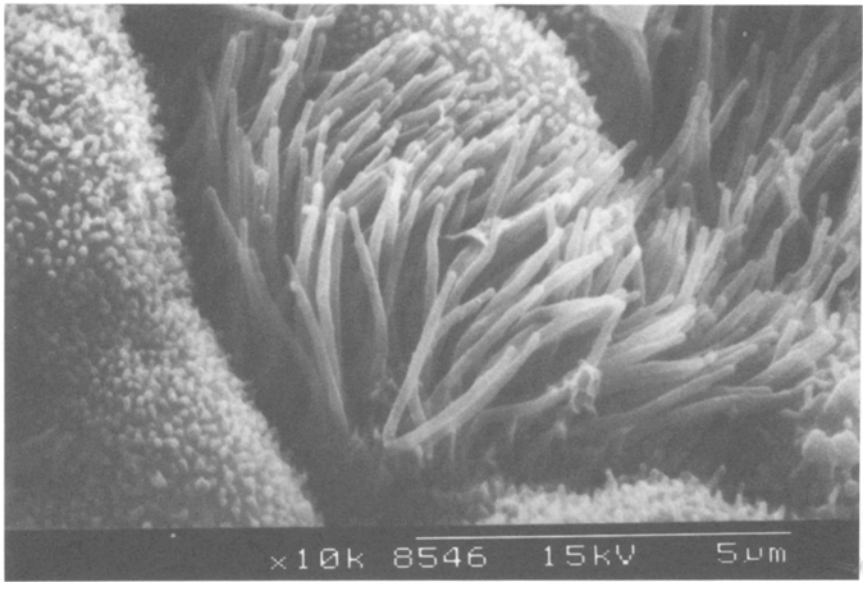

Fig. 8. Trachea of *M. musculus* from control area. Secretory cell and ciliated cell. (Scanning electron microscope)

the mean intercellular distance was 7.8 μm and the percentage of ciliated cells was 53%, whilst in animals from polluted area the values were 6.3 μm and 29%, respectively.

The thickness of pseudostratified tracheal epithelium was about 15.4 μm in animals from the polluted area and 19.2 μm in control animals. In animals from the polluted area a large number of glycoprotein-mucopolysaccharide vesicles and secretory granules were observed all along the cytoplasm. The secretory granules were fairly big, 1.15 μm. Control animals did not show vesicles and there was a moderate quantity of small secretory granules (0.5 μm). The distribution and packing of the nuclear chromatin followed the same pattern (Table 4).

Statistical Analysis

Differences between control and exposed groups were analyzed by Chi-squared test (using the values obtained in each group).

Small Mammals

Apodemus sylvaticus

There were statistically significant differences when comparing types of cells and chromatin in each group, although these differences were not strong (Cramer contingency coefficient). The level of significance chosen to reject the null hypothesis was $P < 0.05$.

Mus musculus

There were statistically significant differences when comparing types of cells in each group ($P < 0.05$). Differences in chromatin were statistically significant at $P < 0.20$.

Passerine Birds

Emberiza cia and *Parus major*

There were no statistically significant differences when com-

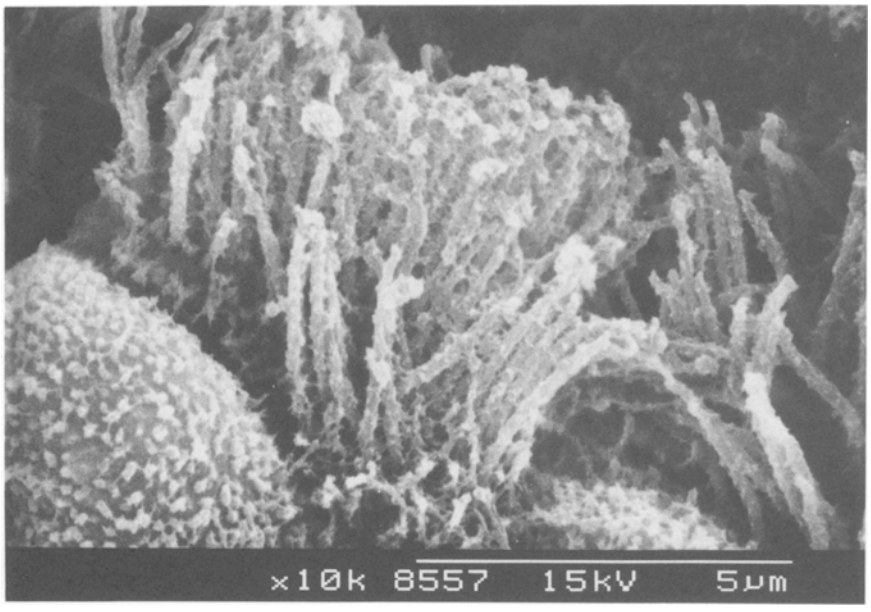

Fig. 9. Trachea of *M. musculus* from polluted area. Secretory cell and ciliated cell covered with mucus. (Scanning electron microscope)

paring types of cells in each group (level of significance was $p < 0.05$). Differences were significant at $P < 0.80$ (*Parus major*) and $P < 0.90$ (*Emberiza cia*).

Carduelis carduelis

We found statistically significant differences only at $P < 0.20$.

Discussion

The effects of various gases on three passerine species show differences according to their location and behavior. *Carduelis carduelis*, in captivity, cannot avoid the gases emitted by the coal-fired power plant, the concentrations of which can vary during the day. The effects on tracheal epithelium are much more strong than those observed in *Emberiza cia*. In spite of the relative sedentary behavior of *Emberiza cia*, it moves far enough away to avoid atmospheric air pollution. There is no mucus and the number of secretory and ciliated cells are similar. But a decrease in ciliar length and a different distribution of secretory granules and vesicles are observed. The mean size of the vesicles and secretory granules is greater, and they are more disorganized. This may be explained in terms of the lubricant and cleaning work of the cilia to eject particles and dust from the trachea.

During the day *Parus major* moves over a wide territory, thus moving in and out of the polluted area. Differences between the control and polluted site were found only when samples were observed by electron microscopy (T.E.M.), i.e., thin mucus stratum.

The hazardous effects of atmospheric air pollution on small wild mammals (*Apodemus sylvaticus*) are mitigated because of their habitat and mobility. Residence in burrows during much of the day results in less exposure to the gaseous pollutants. This may account for the smaller effect on the tracheal epithelium of mammals when compared to birds.

These results agree with the laboratory studies performed by Kawakami (1989) who observed disintegration and sloughing of epithelium in rats exposed to 50 ppm NO_2, and those of Benjebria (1990), Man (1986) who observed exfoliation of ciliated cells after inhalation of several atmospheric pollutants. Due to the solubility of gases in water and that mucus has 95% of water acid components inside the mucus are formed, Miura (1984) who observed degeneration on airway respiratory cells, Savic (1987) who observed that workers exposed to SO_2 inhalation developed episodes of coughing, irritation in eyes, nose and throat, and Scanlon (1987) who observed that dogs exposed to 15–50 ppm SO_2 developed hypersecretion of mucus, sloughing of tracheal epithelium and increment of secretory glands.

A study is in progress to evaluate the effects of the gases emitted by the coal-fired power plant at different concentric distances from the plant.

Conclusions

Having tested various samples of vertebrate fauna in an atmosphere which is rich in SO_2 and NO_x, and according to the atmospheric composition values provided by FECSA (the electricity company) we can establish the following conclusions:

Passerine Birds

1. They presented a marked alteration of the tracheal epithelium and increase in the number of secretory cells compared with ciliated cells.
2. There was a considerable decrease in the length of the cilia.
3. A uniform, thick layer of mucus was observed covering the cilia.
4. The quantity and size of the glycoprotein-mucopolysaccharide vesicles increased in the secretory cells. The secretory granules appeared more dispersed in the cytoplasm.
5. These effects were not observed using light microscopy in *P. major*, but there was a layer of mucus covering the cilia when observed by electron microscopy (T.E.M.)

Small Mammals

1. The specimens from polluted areas showed some alteration of the tracheal epithelium mucus and the percentage of

ciliated cells was greater in polluted specimens compared with control animals.
2. The secretion of mucus was more abundant and almost homogeneous, covering the ciliar stratum. In some cases the thickness became considerable.
3. The cilia were shorter, although the presence of mucus (in some cases very abundant) was marked, and this would provoke an apparent shortening of ciliar length.
4. The animals from the polluted area showed a large number of glycoprotein-mucopolysaccharide vesicles, but this was not observed in control animals.
5. Differences in the quantity and distribution of secretory granules: in control animals these granules appeared crowded together in a large vesicle, but in some sections they were not abundant. In contrast, in animals from the polluted area there were a large number of secretory granules dispersed in the cytoplasm.

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