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Abnormal Cilia in the Bronchial Mucosa

Case Reports of Non-Smoking Women with Bronchogenic Carcinomas and an Experimental Model in Guinea-Pigs

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Summary. Atypical cilia in the bronchial mucosa of non-smoking women and in guinea-pig lungs were studied by the electron microscope. In human cases, numerous compound cilia were observed in the main bronchi. The largest one contained about 40 axial filament complexes in a ciliary shaft. Occasionally, atypical basal bodies were also seen.

In the experimental model in guinea-pigs, 50% oxygen at one atmospheric pressure damaged the bronchial surface and resulted in degeneration and reduction of cilia and pellicular structures. The injury was not sufficiently severe to initiate adaptation and reparative mechanisms in the bronchial mucosa, and rapid renewal of the surface structures was found.

The human cases were associated with bronchogenic carcinoma but the experimental model suggested that atypical cilia were not always related to pulmonary carcinogenesis.

Key words: Compound cilia — Axial filament complex — Bronchitis — Ultrastructure.

The fine structure of kinocilia was first examined by Fawcett and Porter (1954). Today, it is widely accepted that each kinocilium has a constant number of filaments within a single ciliary shaft. Although 9+0, 9+1 and 9+3 patterns for the normal kinocilium are described, most of the kinocilia have two central filaments surrounded by nine peripheral ones with a double structure — 9+2 pattern of the axial filament complex (Rhodin, 1966; Afzelius, 1969; see Konradova, 1973). In the respiratory organs of vertebrates, ciliated epithelial cells have numerous kinocilia showing the 9+2 pattern which is thought to be very stable.

Atypical cilia were first reported by Stair (1962). Following this a number of reports on abnormal cilia in various kinds of animals were presented. Reports on abnormal cilia in the respiratory tract were curiously rare considering the great amount of electron microscopic work carried out, although in man, atypical cilia were noted in a case report by Ailsby and Ghadially (1973) and in an electron microscopic study on the bronchitic bronchus by Watson and Brinkman (1964). Atypical cilia in the middle ear mucosa (Kawabata and Paparella, 1969), the normal endometrium (Hando et al., 1968), the gastritic mucosa (Watanabe et al., 1974) and some benign or malignant tumors (Gaito et al., 1965; Luse and Vietti, 1968) have also been described in man.

In animals, reports of atypical cilia in the middle ear mucosa of the guinea-pig (Kawabata et al., 1969), the thymic cysts of the nude mouse (Cordier, 1974), the tracheobronchial mucosa of the rabbit (Konradova, 1973) and the hamster (Harris et al., 1974) have been published.

One of the most common pathological alterations of tracheobronchial epithelium is squamous metaplasia. Experimentally induced squamous metaplasia of the lung in vitamin A deficient rats (Wong and Buck, 1971) and in dogs in experimental cigarette smoke inhalation (Frasca et al., 1968) were examined with electron microscopy. Only a few intracytoplasmic cilia were found in squamous metaplasia.

This paper reports the presence of abnormal cilia in the bronchial mucosa in non-smoking patients with bronchogenic carcinomas, and both in guinea-pigs treated with soluble immune complex and controls kept in an oxygen rich atmosphere for a long period.

The main aim of this study is an attempt to clarify the nature of the submicroscopic alterations of bronchial mucosa caused by environmental factors other than cigarette smoke and the relationship between these morphological findings and initial changes of tracheobronchial pathology.

I. Case Reports of Human Cases

Short Histories

Case No. 1.: The patient was a 48 year old housewife, a non-smoker. She was informed the presence of an abnormal shadow in the left upper lobe of the lung and was admitted to the National Kanagawa Hospital where she was found to have a bronchogenic carcinoma of the lung.

Case No. 2.: A 58 year old clerk, also a non-smoker. About 28 years ago, she was treated for pulmonary tuberculosis and was followed up with chest X-rays. These eventually showed an abnormal shadow in the right lower lobe. Bronchial brush cytology revealed an adenocarcinoma of the lung.

The two patients had no history of preoperative irradiation or administration of antitumor drugs, nor of specific occupational or environmental conditions which might constitute a hazard.

Materials and Methods

Specimens for electron microscopy were obtained from tumor masses, peripheral lung tissues and the resected ends of the bronchi. These specimens were fixed with 1% osmium tetroxide in a phosphate buffer for 2 hours soon after the lungs were removed and were dehydrated with graded alcohols and embedded in epoxy resin. Ultrathin sections were cut with glass knives on a Porter-Blum ultratome, and stained with uranyl acetate and lead. They were examined with a JEM IOOB electron microscope. The remainder of the lungs was fixed with 10% formalin solution and prepared for routin histological examination.

Observation

Case No. 1: The tumor was an adenocarcinoma of the lung. Main bronchial epithelium appeared somewhat hyperplastic but no squamous metaplasia was seen. Electron microscopy revealed the presence of abnormal cilia in the bronchial mucosa. These were mostly found in the main bronchi and rarely in the bronchioles.

One of the most characteristic appearances was the so-called compound cilia which contained two or more axial filament complexes within a ciliary shaft. In cross section, the largest had about 40 axial filament complexes within a single ciliary shaft. In longitudinal section, ciliary bodies were irregularly arranged and were found at abnormal distances from the cell surface, occasional intracytoplasmic cilia were observed. In addition, irregular arrangements and changes in numbers of the axial filaments were commonly seen. A swollen cilium with much more intracellular matrix than is usually seen in a ciliary shaft was occasionally observed. Some abnormal ciliary bodies were also noticed (Figs. 1, 2).

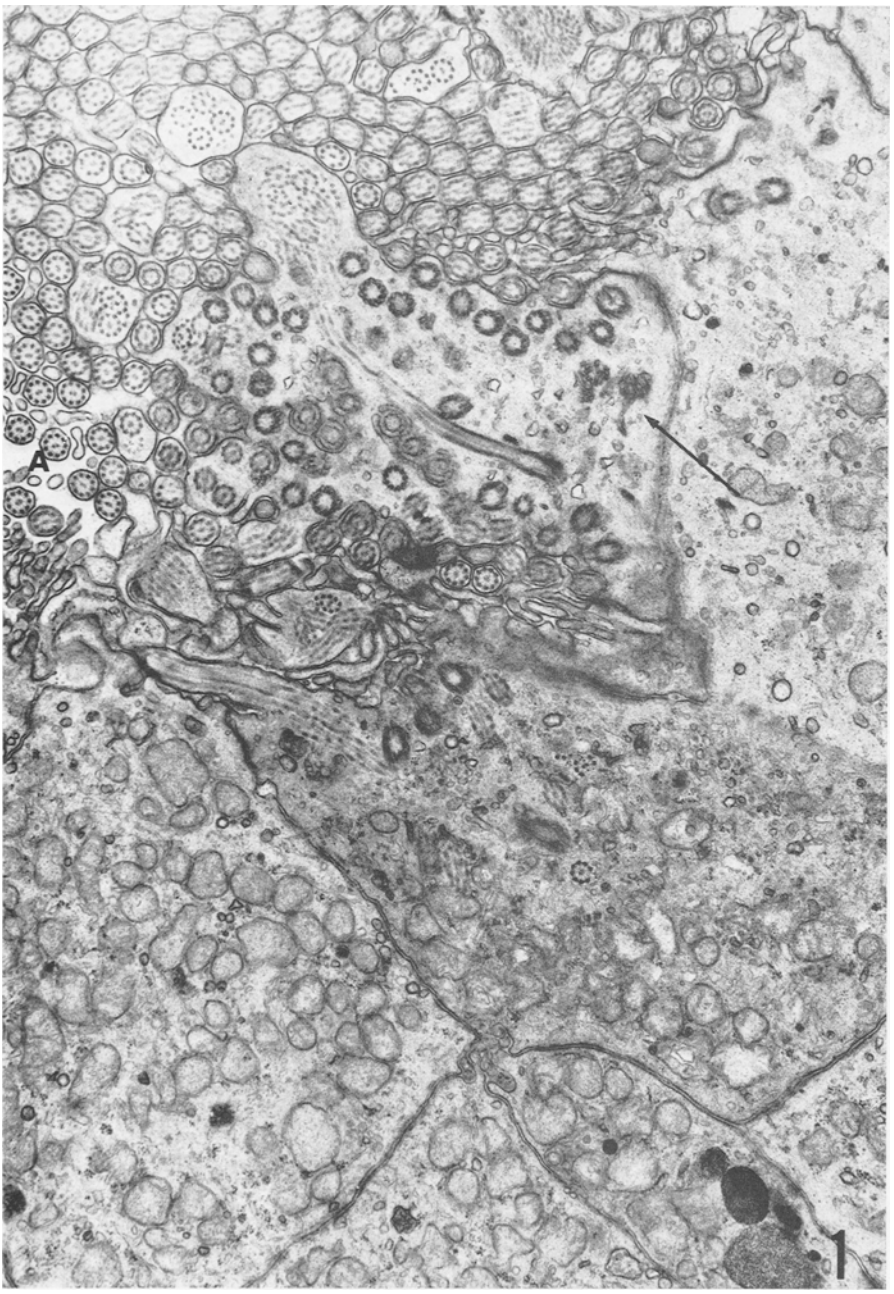


Fig. 1. Abnormal cilia in the bronchial mucosa from the Case I. Numerous compound cilia transversely and longitudinally sectioned are seen. Intracytoplasmic cilium and abnormal basal bodies (arrow) are also observed. × 16,000. A Apical surface; B Basement membrane

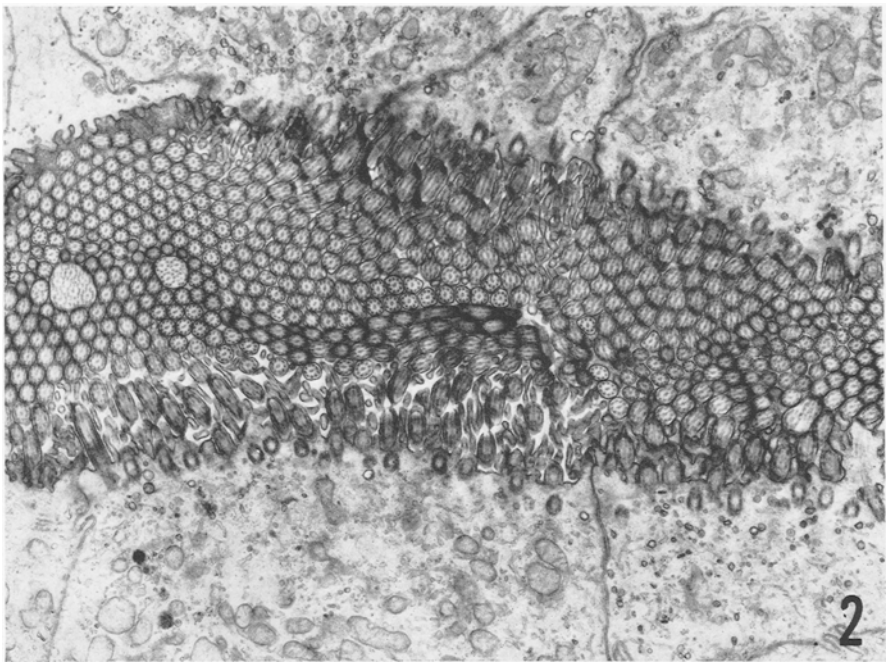


Fig. 2. Compound cilia from the Case 1. Compound cilia containing 2, 4 and 6 axial filament complexes are observed. × 8,800

Case No. 2: Histological examination revealed an adenocarcinoma of the lung located at the right lower lobe and calcified nodules possibly due to old tuberculosis. The bronchial epithelium appeared almost normal in light microscopic examination. Electron microscopic observations revealed the presence of abnormal cilia but they were few in number. Compound cilia containing three axial filament complexes and disorientated axial filaments were occasionally observed.

II. Experimental Model in the Guinea-Pig Lungs

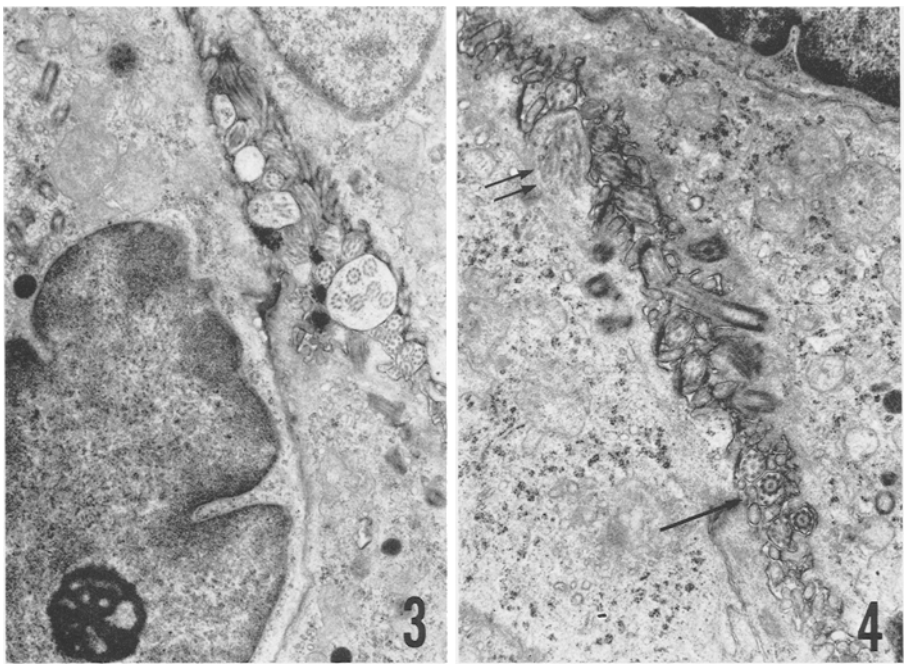
Materials and Methods

Conventional guinea-pigs, weighing from 200 to 250 g and of both sexes were used. The animals were injected with 1 ml of soluble BSA anti-BSA rabbit serum complex intravenously and were kept in room air or 50% oxygen at one atmospheric pressure for one to 150 experimental days.

Non-treated animals kept in room air or 50% oxygen were also kept for the same period. The lungs and other organs were prepared for light and electron microscopic observation by the procedures normally used in the laboratory.

Observations

After several days, interstitial pneumonitis occurred in all the soluble immune complex injected animals. The relatively high oxygen condition caused the persistence of inflammatory processes and resulted in diffuse pulmonary fibrosis similar to that seen in man (Torikata, 1974). In the late stage, the bronchial epithelium showed hyperplasia but no squamous metaplasia was observed in this



Figs. 3 and 4. Compound cilia of the guinea-pig bronchus from the 10th day. Compound cilia in cross section (Fig. 3) are very similar to those in Figs. 1 and 2. An abnormal cluster of basal bodies in a ciliated epithelial cell is observed. In longitudinal section (Fig. 4), irregular arrangement of numerous axial filaments in a ciliary shaft is observed. A kinocilium (arrow) contians two additional filaments in an envelope. × 8,500 (Fig. 3) × 11,500 (Fig. 4)

experimental series. On the 10th day, abnormal cilia were frequently found. Compound cilia containing five or more axial filament complexes were observed and their morphology was very similar to that of the human cases (Figs. 3, 4). Intracytoplasmic cilia in the oedematous cytoplasm were also observed (Fig. 5). In the lungs, abnormal cilia were still observed in the bronchial mucosa after the 100th day (Fig. 6).

Non-treated guinea-pigs kept in the oxygen rich atmosphere showed the same bronchial pathology. In animals kept under hyperoxygenic condition, epithelial hyperplasia was noted after 30 or more experimental days and atypical clusters of the basal bodies and intracytoplasmic canaliculi covered with cilia and microvilli were occasionally observed (Fig. 7). Among these cilia, small numbers of atypical cilia were also observed. The incidence of abnormal cilia in animals not subjected to oxygen inhalation was very low. Only two abnormal cilia in one soluble immune complex injected animal were found. One was a compound cilium containing two axial filament complexes and the other had an abnormal filament of 10+2 pattern. The abnormal cilia found in the human cases, and in the experimental animals are summarized in Table 1.

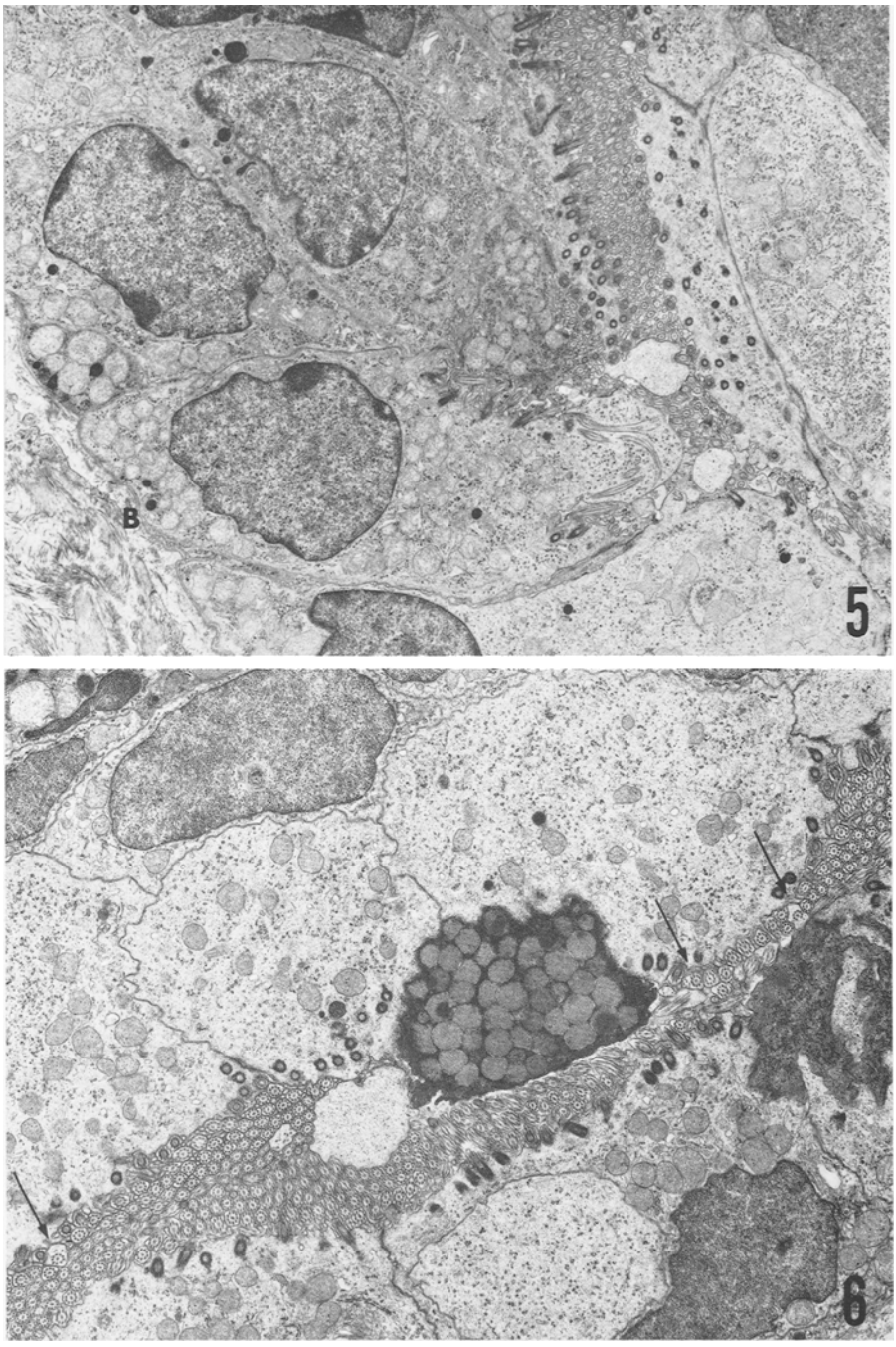


Fig. 5. An electron micrograph of the guinea-pig bronchus from the 10th day. Edematous cytoplasmic protrusions and some intracytoplasmic cilia are observed. × 4,900

Fig. 6. An electron micrograph of the guinea-pig from the 100th day. Basal bodies are regularly arranged at the apical portion but abnormal cilia are still observable (arrows). × 6,000

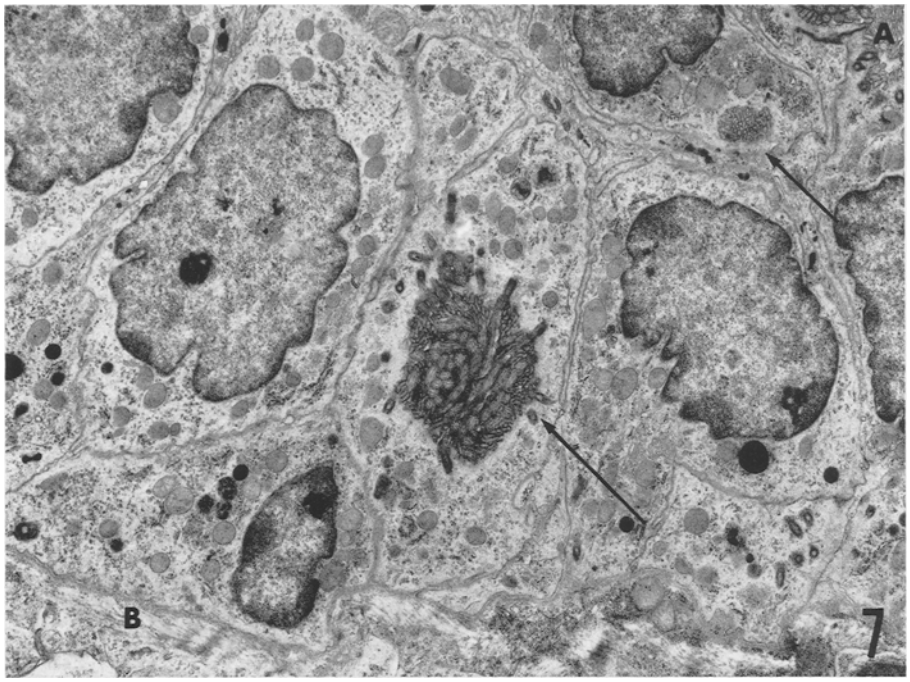


Fig. 7. Intracytoplasmic canaliculi covered with cilia and microvilli in the guinea-pig bronchus from the 80th day. × 5,000

Table 1. Atypical cilia in human cases and in guinea-pigs

	Single axial filament complex				Compound cilia ( )*
	changes in		swollen cilium	intra-cytoplasmic cilium	
	numbers	arrangements			
Human cases					
Case No. 1. 48 y.o. F	+	++	+	+	+++ (40)
Case No. 2. 58 y.o. F	++	+	+	−	++ (3)
guinea-pigs					
1. immune complex injection	+	−	−	−	+
2. 50% oxygen inhalation	++	++	++	+	+ (3)
3. 1. plus 2.	++	++	++	++	++ (8)
4. control	−	−	−	−	−

−not seen; + observed but rare; ++ occasional; +++ numerous: ( )* maximum of the axial filament complexes

Discussion

We have found abnormal cilia in non-smoking women with bronchogenic carcinomas and in guinea-pigs subjected to increase concentrations of inhaled oxygen.

Sorokin (1968) described ciliogenesis in fetal rat lungs but there are few papers reporting repair processes in bronchial mucosa covered with ciliated epithelial cells. Although abnormal ciliogenesis is partially related to genetic factors, abnormal cilia in the respiratory organs are usually caused by environmental factors. These morphological changes may cause inadequate ciliary propulsion and may result in prolonged attachment of carcinogens to the bronchial epithelium. If this hypothesis is accepted, it is interesting to note that abnormal cilia and irregular ciliary beats are related to carcinogenesis in the respiratory organs.

Hando et al. (1968) reported the presence of numerous abnormal cilia in the human endometrium and suggested that abnormal ciliogenesis was due to rapid renewal of the surface epithelial cells. Watanabe et al. (1974) reported the presence of abnormal cilia in inflamed human gastric mucosa. These reports suggest that abnormal ciliogenesis is related to cell repair and proliferation.

Harris et al. (1974) reported the presence of a closed intracellular space covered with cilia far from the cell surface in hamster lungs which had been exposed to carcinogens. In this study, intracytoplasmic canaliculi covered with numerous cilia and pellicular structures were also observed in the experimental animals. These findings were interpreted as part of the recanalization process of a bronchus filled with hyperplastic undifferentiated epithelial cells.

Harmful effects on smooth muscle cells by soluble immune complexes were studied by Trapani et al. (1958) and in our previous papers, the pathogenetic effects of BSA-anti BSA rabbit serum complex on guinea-pigs were reported (Yamaguchi et al., 1972, 1973). In this study, effects of soluble immune complex on the bronchial smooth muscle cells were not seen and the induced changes in bronchial epithelium were negligible.

The toxic effects of oxygen were described by Smith in the 19th century (1899). Today, it is well known that pathological effects are related to the partial pressure of the gas and that 60% oxygen at one atmospheric pressure is the upper limit of harmlessness for the normal lung. In this experimental series, 50% oxygen caused no pathological changes in the alveolar linings of the control animals (Torikata, 1974) but surface structures of the bronchial epithelia were damaged.

These morphological alterations of the kinocilia are thought to be the initial changes of bronchitis. In human cases the mechanism of atypical ciliogenesis is not clear but in some conditions, cigarette smoke, irritating gases and air pollutants might play a major role in abnormal ciliogenesis.

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