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Goodpasture's syndrome in ageing. An experimental study on the rat. II

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Summary. The following hypothesis is suggested: if a lung disease is induced in an adult rat, then its lung, in the senile age, will be different from what is considered to be a normal senile lung. In order to demonstrate this, the pulmonary state of two groups of old rats, one of which had suffered from Goodpasture's syndrome in the adult age has been assessed morphometrically.

Fifty-three Wistar rats were used. They were divided into two groups: 1) healthy old rats; and 2) diseased old rats. Antipulmonary serum was administered to the latter and they were sacrificed a year later. Making use of a computing system, we calculated the following data for each group: the alveolar chord length, the alveolar wall thickness and the surface of the bronchial-associated lymphoid tissue (BALT). The alveolar macrophages (AM) with haemosiderin which were found in the lung tissue were also counted and the percentage of goblet bronchial cells and that of bronchoalveolar lavage (BAL) cells was also assessed.

From the results, the following points should be emphasized: in diseased old rats, an increase in the alveolar chord, a decrease in lymphocytes with an increase in the AM of the BAL, and a decrease in goblet cells and AM with haemosiderin occur, all of which are significant when these rats are compared to healthy old rats.

From this experiment it can be inferred that the lungs of the diseased old rats are morphologically different from those of the healthy old rats; the findings cannot be related to an inflammatory process.

Key words: Lung, Old, Morphometry, Rat

Introduction

Goodpasture's syndrome is characterized by a lung haemorrhage with glomerulonephritis and anti-basal membrane circulating antibodies (Carré et al., 1988). The onset of this disease lies in the fact that the organism creates antibodies to a type IV collagen component which is a specific component of the basal membranes (Wieslander et al., 1983). Since 1900, when Lindemann administered antikidney heterologous serum to an animal for the first time, Goodpasture's syndrome has been induced to laboratory animals in many experiments. Different experimental models have been described as follows: lung affection; renal affection; with heterologous antibodies; with homologous antibodies; etc (Shigematsu and Kobayashi, 1972; Steblay and Rudofsky, 1983).

Until the beginning of the last decade it was considered that Goodpasture's syndrome brought on fatal consequences for the human being and on many occasions, a lung problem was the cause of death (Bergrem et al., 1980). Nowadays, survival for years is being achieved in 50% of these patients (Travis et al., 1990). Experimentally speaking, the evolution of this disease has been described only for the first fifteen days, a period of time which corresponds, in the heterologous experimental Goodpasture's syndrome, to the forming of antibodies against the anti-basal membrane serum administered (Lan et al., 1991).

Prompted by the lack of knowledge of the possible sequels of this disease in the senile age, we present this paper, in which the following hypothesis is suggested: heterologous Goodpasture's syndrome, when experimentally induced in the rat, will provoke considerable modifications in the lung in the senile age. In order to demonstrate this, adult rats to which anti-alveolar basal membrane serum was administered were used. The animals treated in this way were sacrificed a year later and their lungs were morphometrically studied and compared to those of the old rats which had not been treated.

Materials and methods

Fifty-three healthy male Wistar rats were used: their average age was 23 months and they were divided into two groups as follows:

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1) Healthy old rats: Thirty old animals to which no substance was administered.

2) Diseased old rats, one year old: Twenty-three animals to which antipulmonary serum was administered in their adult age and which were sacrificed a year later.

Antipulmonary serum

The antipulmonary serum was obtained from the rabbit (Meezan et al., 1975; Jenning et al., 1981) and was generously provided by Laboratorios Operon®. Rat antipulmonary rabbit serum was administered by intravenous injection at the rate of a daily dose for three days.

Sacrifice

All the animals studied in this experiment were sacrificed. For this purpose, they were anaesthetized with Nembutal® 1% (1ml/100 gr) by intraperitoneal injection. Broncho alveolar lavage (BAL) was performed on the right lung. The left lung was fixed by immersion in 10% formalin with tracheal insufflation of the same fixative at a positive pressure of 26 cm of water, in order to carry out a histological study. The sections were contrasted using the PAS-Alcian-Blue and ferrocyanide methods.

BAL

This was carried out in accordance with the technique, already described (Escolar Castellón et al., 1991), which made it possible to obtain a percentage count of lymphocytes, polymorphonuclear leukocytes (PMN) and alveolar macrophages (AM).

Histological study

A morphometrical study was performed, according to our own technique, as already described (Escolar Castellón et al., 1991, 1992). For this purpose, aleatory sampling was carried out and the variables were

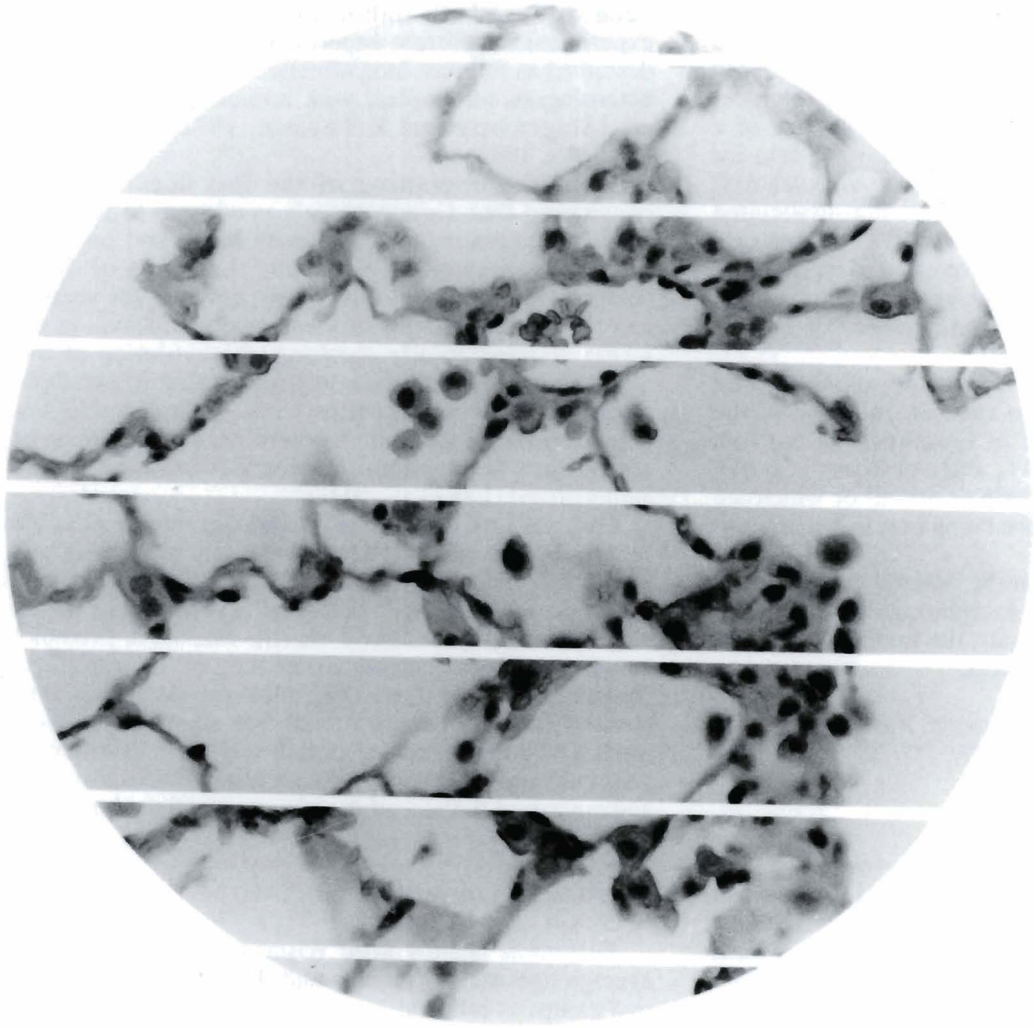

Fig. 1. Overlap of the seven straight lines in a histological field in order to obtain the alveolar chord distance. PAS-Alcian-Blue staining. x 200

classified into alveolo-interstitial, broncho-epithelial and BALT.

Alveolo-interstitial variables: In the preparations contrasted with the PAS-Alcian-Blue method, two variables were quantified: 1.- Alveolar chord length (Fig. 1); 2.- Mean alveolar wall thickness. Both are expressed in µm.

In the sections which were stained using ferrocyanide, a percentage count of AM with the blue vesicles was performed (Fig. 2).

Bronchoepithelial variable: This was quantified using PAS-Alcian-Blue staining; it is presented as the percentage of goblet cells. It was obtained by linking all the PAS-Alcian-Blue positive cells to all the nuclei of the epithelial cells (Fig. 3). The count took place at bronchial levels, I, II and III, as suggested by Olesen et al. (1987).

Variables of the bronchial-associated lymphoid tissue (BALT): Two variables related to the BALT (Figs. 4, 5) were quantified using the PAS-Alcian Blue method: 1.- Lymphatic area (LA): this is defined as the surface of BALT in the section; it is expressed in mm^2. 2.- Lymphatic epithelium (LEp): this is the length of the flat bronchial epithelium, which corresponds to the BALT; it is expressed in mm.

The computer programme used for the quantification were devised by ourselves, with the exception of Mac Draft®.

Statistical study

All the data are expressed as mean ± SEM; for the distribution study, the Kurtosis and Skewness indices were used and values of between ±1 were taken as normal distribution values. When the values of the results of the variables neared normal distribution (which only occurred in the case of the alveolar chord length and alveolar wall thickness), they were compared to the Student-T parametric test; the variables whose results were far from normal distribution were compared by means of the Mann-Whitney U non-parametric test. Probability values lower than 0.05 were considered significant in all cases. The statistical study was performed on a Macintosh® II cx with the StatView® II

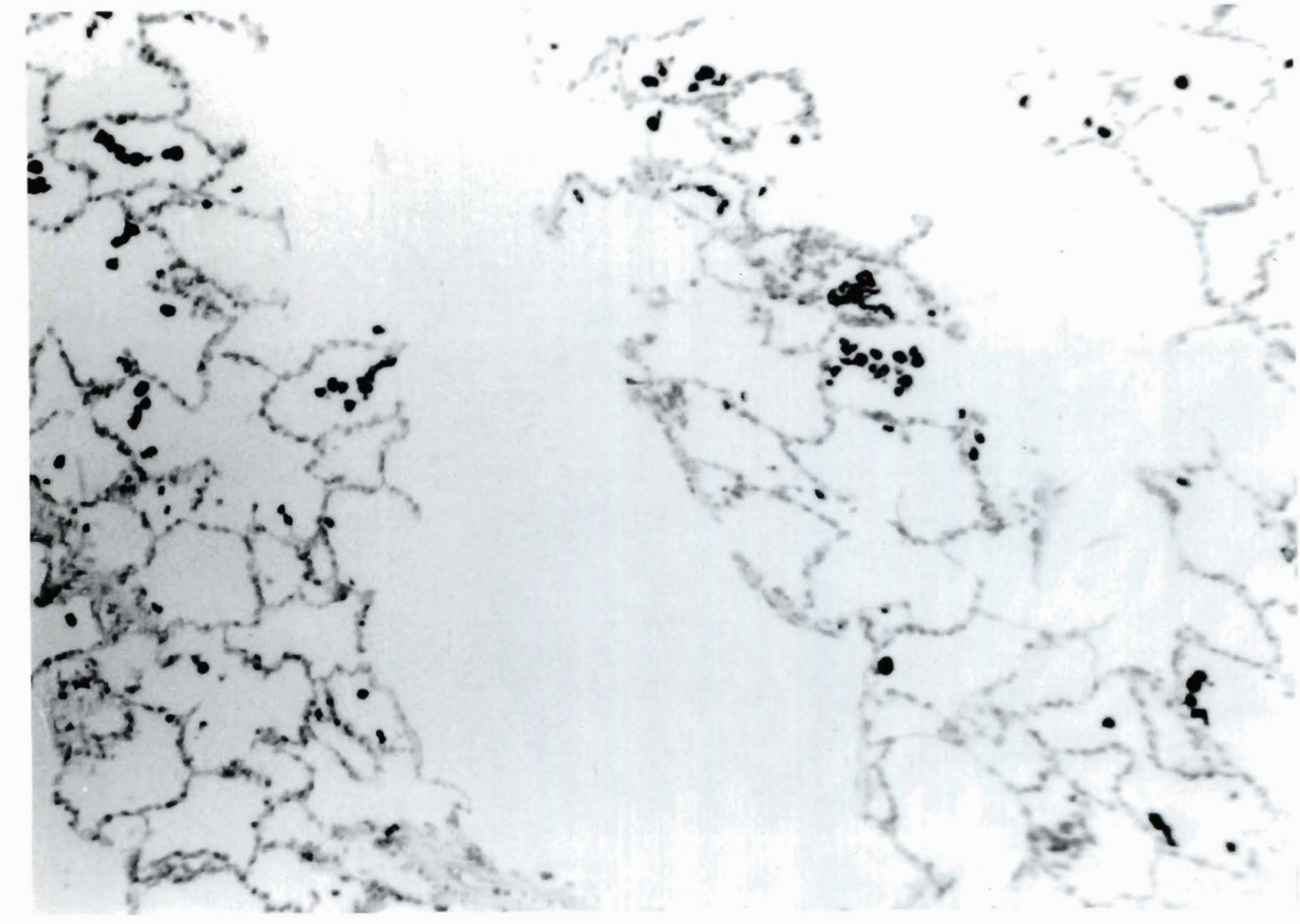

Fig. 2. Alveolar macrophage with haemosiderin. Ferrocyanide and neutral red staining. x 60

programme.

Results

BAL (Table 1)

A year after the administration of antipulmonary serum to the rat, a percentage increase in alveolar macrophages (72.57 ± 23.99) and a decrease in lymphocytes (22.64 ± 12.17) were recorded, all of which was significant ($p < 0.01$) when compared with the group of untreated animals (alveolar macrophages: 54.37 ± 19.75; lymphocytes: 36.78 ± 15.34).

Alveolo-interstitial variables (Table 2)

The alveolar chord length increased significantly ($p < 0.001$) in the animals to which antipulmonary serum had been administered (43.08 ± 8.66) in relation to the animals which had not been treated (36.87 ± 10.30), while there were no significant differences found in the alveolar wall thickness between the two groups of animals used. The quantification of the haemosiderin; i.e. ferrocyanide-positive cells, displayed significant differences ($p < 0.001$) between the two groups of animals used; those which had been treated with antipulmonary serum revealed fewer ferrocyanide-positive alveolar macrophages (13 ± 29.98) in relation to the control group (44.39 ± 20.79).

Bronchial variables (Table 2)

The percentage of goblet cells in the bronchial epithelium decreased significantly ($p < 0.05$) in the animals to which antipulmonary serum had been administered (13 ± 29) when these were compared with the untreated animals (53.39 ± 10.89). None of the variables which define the BALT (lymphatic area and

Table 1. Mean ± standard deviation of values obtained in broncho-alveolar lavage variables.

GROUP	LYMPHOCYTES	POLYMORPHO-NUCLEARS	ALVEOLAR MACROPHAGES
Healthy old rats	36.78±15.34	8.83±18.60	54.37±19.75
Diseased old rats	22.64±12.17*	4.44±16.11	72.57±23.99*

*: $p < 0.01$ when group of diseased old rats is compared to that of healthy old rats.

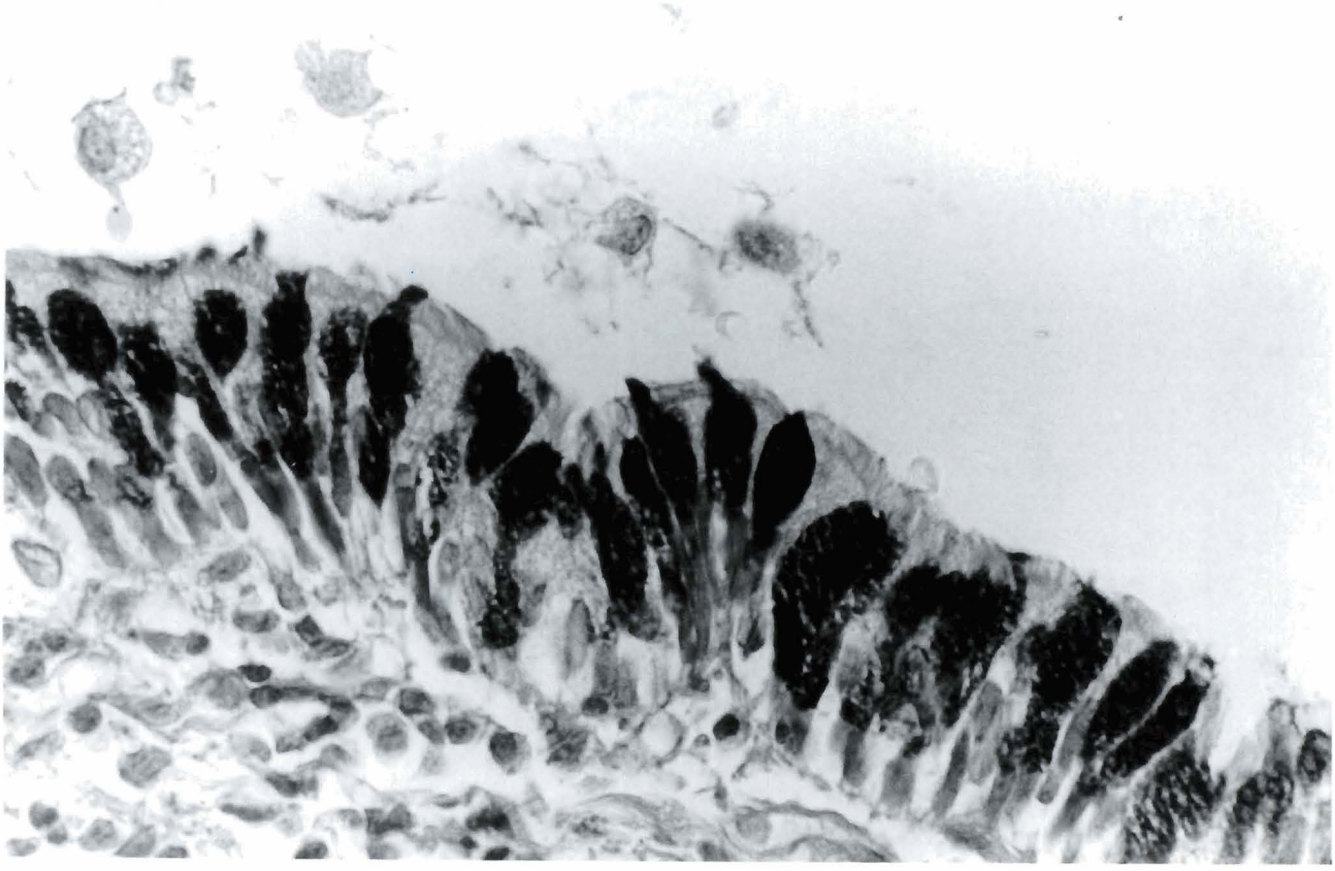

Fig. 3. Bronchial epithelium with goblet cells (arrow). PAS-Alcian-Blue staining. x 400

lymphatic epithelium) showed significant differences between the two groups of animals used.

Discussion

Our results support the suggested hypothesis since the mortality which appears in the diseased old rat group, together with the differences found in the lung between the two groups used, lead us to consider that the group of old rats to which a lung disease was induced have their own characteristics, which are different from those of the healthy old rats.

The previous models of experimental interstitial lung disease are characterized by the fact that they display a percentage increase in lymphocytes both in the BAL, and occasionally in the PMN, with the consequent decrease in AM (Escolar Castellón et al., 1991). In our model of the diseased old animal we have found quite the opposite; namely a significant decrease in lymphocytes and an increase in AM. AM are mediating cells which are essential to the immune alveolar response (Crystal et al., 1984) and play an important role in Goodpasture's syndrome (Lan et al., 1991). As our BAL study is based on a percentage cell count, one cannot be sure that the results reflect, in terms of absolute values, an increase in AM, a decrease in lymphocytes or both.

The emphysematous lung is characterized by the fact

Table 2. Mean ± standard deviation of the values obtained in the histological variables.

GROUP	ALVEOLAR CHORD	WALL THICKNESS	HAEMOSIDERIN	GOBLET CELLS	LYMPHATIC AREA	LYMPHATIC EPITHELIUM
Healthy old rats	36.87±10.30	17.45±9.08	44.39±20.79	53.39±10.89	1.26±1.21	1.57±1.19
Diseased old rats	43.08±8.66*	13.44±9.53	13±29.98*	13±29**	2.09±4.32	1.72±1.24

*: p< 0.001 when group of diseased old rats is compared to that of healthy old rats; **: p< 0.05 when group of diseased old rats is compared to that of healthy old rats.

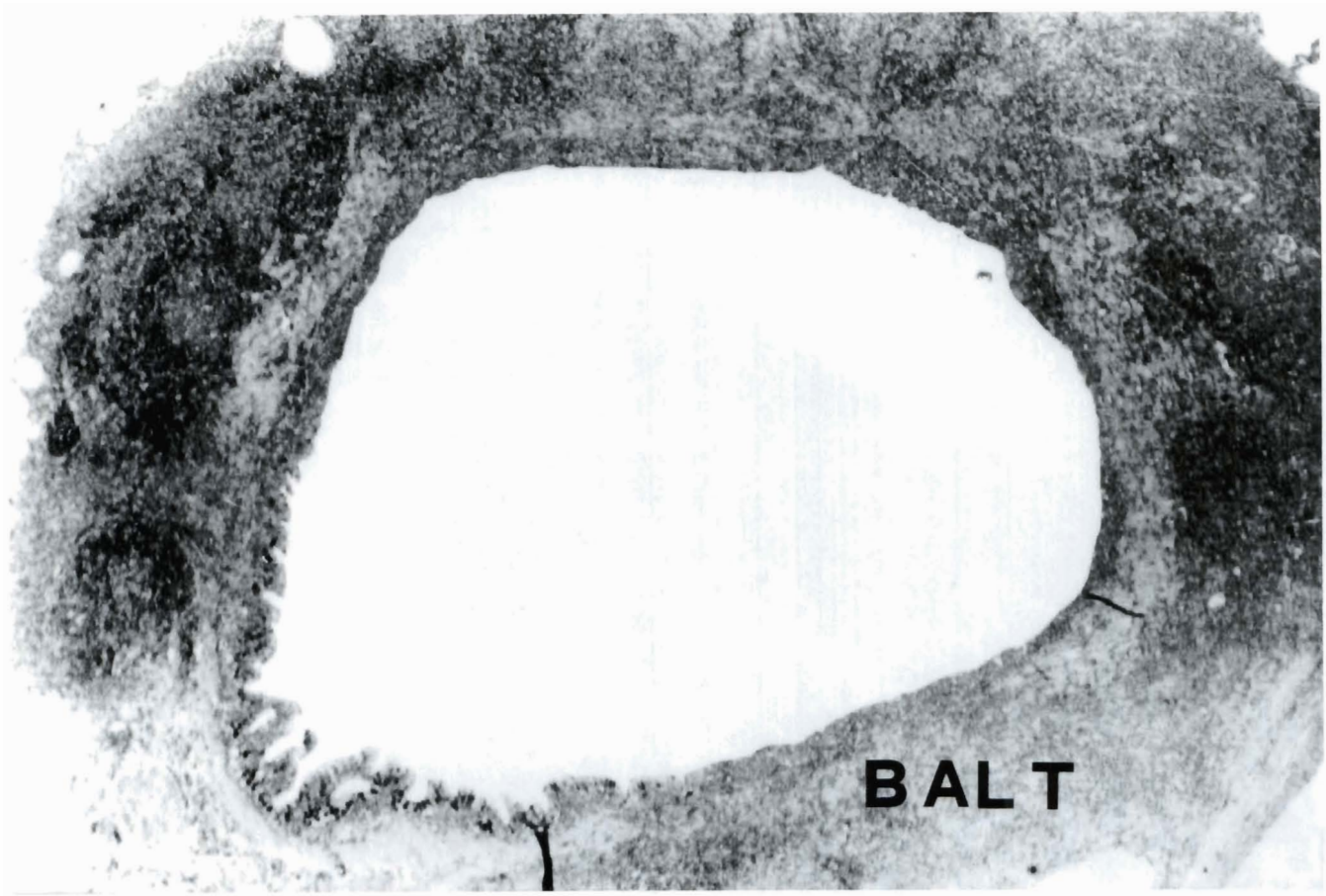

Fig. 4. Bronchial-associated lymphoid tissue. Lymphatic epithelium. PAS-Alcian-Blue staining. x 400

that it displays dilatation of the air spaces with loss of the interstitial component in the distal portion of the lung (Snider et al., 1985); the senile lung coincides with the emphysematous lung in the alveolar dilatation and differs from it in the fact that the former maintains in the interstitial component (Pinkerton et al., 1982). The results obtained after measuring the alveolar chord length lead one to consider that the size of the alveolus of the diseased old rats increased in relation to the healthy old rats and the wall thickness was maintained. Taking into account the fact that the destruction of the alveolar wall in diseased old animals has not been proven, the model obtained could be similar to the senile lung. It is necessary to emphasize two points: 1) the lung of healthy old rats is a senile lung, that is to say, its alveoli are dilatated, when compared to that of healthy adult rats (Pinkerton et al., 1982); 2) our diseased old rat specimens displayed far greater alveolar dilatation. Therefore, the objectivized alveolar dilatation in diseased old rats is far too great for us to consider the lungs of these animals as being normal senile lungs.

Alveolar inflammation displays an increase in alveolocapillary permeability (Mishkin et al., 1987), which can be objectivized by demonstrating the direct or indirect presence in the alveolar compartment of substances of high molecular weight, which are usually found only in the blood. The decrease in the number of macrophages with haemosiderin in the animals which were treated leads us to believe that the transfer of the haemoglobin through the alveolocapillary membrane was limited in the animals treated. This fact is opposed to the possibility of the existence of an inflammatory process in our diseased old animals.

In general terms, bronchial inflammation is characterized by hyperplasia of goblet cells, which produce mucus (Bates, 1973; Olesen et al., 1987). In our case the animals treated have shown a decrease in the percentage of goblet cells. Although the studies carried out on the bronchial epithelium, both in human beings and rats, coincide in the fact that the proportion of «goblet-cells/non-secretory cells» varies along the respiratory tract (Olesen et al., 1987; Saetta et al., 1989; Södeberg et al., 1990), in rats, this proportion tends to be maintained between the same bronchial levels of different lungs (Olesen et al., 1987). This was the factor which led us to study the same bronchial segment in all cases, sections I, II and III according to Olesen et al. (1987). Many factors have been related, in the human being, to the hyperplasia of bronchial goblet cells, such as irritating gases, asthma, etc (Söderberg et al., 1990).

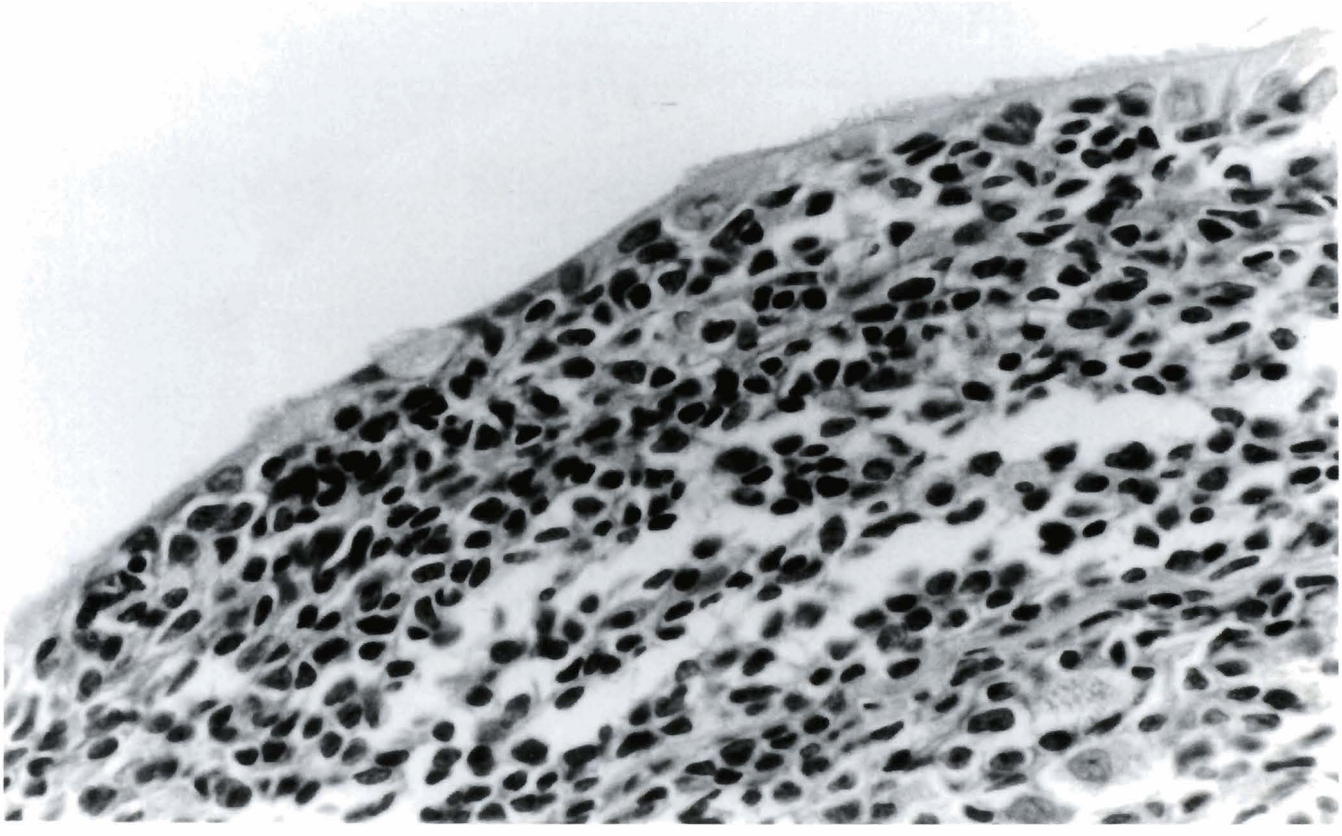

Fig. 5. Bronchial-associated lymphoid tissue. PAS-Alcian-Blue staining. x 25

However, in animal experimentation, it has not always been possible to demonstrate an increase in mucus and/or goblet cells (Wiswell and Wiswell, 1990; Du et al., 1991).

The BALT is a lymphoepithelial organ, composed of an epithelial and a lymphatic part, which proliferates in the face of lung aggression and age (Van der Bruffe-Gamelkoorn et al., 1985; Anderson et al., 1986). The increase in size which takes place during senescence has been linked to the continuous exposure to antigens which occurs throughout life (Anderson et al., 1986). In our experiment the BALT was the only structure which did not display significant variations. As the size of the BALT was similar in both groups of old animals, one must consider that the environmental factors that can influence the proliferation of the BALT which takes place in the senile age are of secondary importance.

By way of summary and in accordance with the data which have been obtained, the lungs of the diseased old rats group cannot be considered as normal senile lungs, because their alveoli were too dilatated, the alveolocapillary permeability was possibly altered, the mucus-producing bronchial cells were fewer and the cytological formula of the BAL was altered. All these data lead us to believe it unlikely that the lungs of the diseased old rats are related to an inflammatory process.

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