

## Studies on the pathogenicity of *Acholeplasma axanthum* in swine

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### SUMMARY

*Acholeplasma axanthum* sp. was isolated from the lung of swine with catarrhal pneumonia. Clinical symptoms of respiratory disease, gross and histological lesions of pneumonia, as well as serological response were produced by intranasal inoculation of 'miniature pigs' with the supernatant of lung suspension containing *Acholeplasma axanthum* and by a 48 hr. broth culture of the strain.

A similar picture of disease was observed in animals held in contact with the animals inoculated with untreated lung suspension. *Acholeplasma axanthum* was isolated from the nasal cavity, lung and peribronchial lymph nodes 7–41 days after inoculation. No lesions were observed after inoculation of pigs with the supernatant of lung suspension pretreated with oxytetracycline or chloroform, and no successful isolation of *Acholeplasma axanthum* could be achieved after this treatment.

### INTRODUCTION

Up to the present the following, still existing *Mycoplasma* and *Acholeplasma* species have been grown from swine: *A. granularum* (Switzer, 1953), *M. hyorhinis* (Switzer, 1955), *M. hyopneumoniae* (*suipneumoniae*) (Mare & Switzer, 1965; Goodwin, Pomeroy & Whittlestone, 1965, 1967), *M. gallinarum* and *M. iners* (Taylor-Robinson & Dinter, 1968), *A. laidlawii* (Taylor-Robinson & Dinter, 1968; Stipkovits, Schimmel & Varga, 1973), *M. hyosynoviae* (Ross & Karmon, 1970), *M. flocculare* (Meyling & Friis, 1972) and *A. axanthum* (Stipkovits, Varga & Schimmel, 1973).

Among these species *M. hyopneumoniae* (*suipneumoniae*) (Goodwin *et al.* 1965), *M. hyorhinis* (Gois, Valicek & Sovadina, 1968), *M. hyosynoviae* (Ross & Duncan, 1970) and *A. laidlawii* (Dzu *et al.* 1971) proved to be pathogenic.

This paper presents data about the recent isolation and studies on the pathogenicity of *Acholeplasma axanthum*.

## MATERIALS AND METHODS

*Animals*

Four- to six-month-old specific pathogen free (SPF) miniature pigs originating from Minnesota and reared on the farm of our institute were used in the experiments.

*Artificial infection*

A 10% suspension prepared from the lung of a conventional pig suffering from catarrhal pneumonia and originating from a farm heavily infected with enzootic pneumonia was used for intranasal inoculation of animals as follows: four animals (nos. 6–9) were given 3 ml. supernatant of untreated lung suspension, two pigs (nos. 16, 17) received 3 ml. suspension pretreated with oxytetracycline (2000 µg./ml.) for 1 hr. at 37° C., two animals (nos. 14, 15) were injected with 3 ml. suspension pretreated with 20% chloroform for 1 hr., two pigs (nos. 10, 11) were infected with 3 ml. of 5-times cloned 48 hr. broth culture of *A. axanthum* (no. 112/BA 3-sc 5/B3) ( $3 \times 10^6$  CFU), which had been isolated from the lung suspension mentioned above. Two animals (nos. 12, 13) were kept in close contact with animals nos. 6–9 injected with untreated supernatant of lung suspension and two animals (nos. 18, 19) were left as controls. Animals were examined for clinical symptoms periodically and for the presence of mycoplasmas in the nasal cavity 1–2 weeks after injection. Experimental pigs were killed at various times after inoculation. Post-mortem examination, including histological studies using haemalum-eosin, resorcin-fuchsin staining and Van Gieson's and Gömöri's techniques was performed, as well as mycoplasma isolation from the nasal cavity, lung and peribronchial lymph nodes.

*Examination for mycoplasma and acholeplasma*

Nasal swabs and various dilutions of lung and lymph node suspensions were inoculated into liquid media described by Hayflick (1965) and by Goodwin *et al.* (1965) for cultivating at least 3 weeks. Growth of micro-organisms was checked by periodical plating. Isolates were cloned 3 times and examined biochemically (Stipkovits *et al.* 1973) and studied in growth inhibition test using the following antisera: *A. granularum* (Friend), *M. hyorhinis* (PG29), *M. hyosynoviae* (AMRC/C104), *M. hyopneumoniae* (NCTC 10127), *M. gallinarum* (PG16), *M. mycoides* var. *mycoides* (PG1), *M. bovis genitalium* (PG11), *M. bovirhinis* (PG43), *M. agalactiae* var. *agalactiae* (PG2), *M. agalactiae* var. *bovis* (Donetta), *A. modicum* (Squire PG49), *M. alkalescens* (PG51), group 7 (Leach PG50), group L (Al-Aubaidi, B 144P), *M. gateae* (CS), *A. axanthum* (ATCC 25176), *M. mycoides* var. *capri* (PG3), *M. pneumoniae* (MAC), *M. fermentans* (PG18), *M. arthritis* (PG6), *M. hominis* (PG21), *M. orale* I (CH 19299), *M. orale* II (CH 20247), *M. salivarium* (PG20), *M. gallisepticum* (PG31).

*Serological examination*

Serum samples collected from animals before and 3 weeks after inoculation (except animals nos. 6 and 7, from which serum was obtained 1 and 2 weeks after inoculation respectively) were examined by the indirect haemagglutination test (Stipkovits, 1964). *Acholeplasma axanthum* culture was sedimented, the sediment was washed three times at 10,000 g for 40 min. in PBS and diluted to one-twentieth of the original volume of broth culture. The antigen was treated ultrasonically for 5 min. (15 amp. MSE. u.P.U.) and used for sensitization of trypsin-treated chicken erythrocytes. The serum samples were adsorbed with chicken erythrocytes before testing.

## RESULTS

*Clinical observations*

Animals (nos. 6–9) inoculated with untreated supernatant of lung suspension and with broth culture of *Acholeplasma axanthum* (nos. 10, 11), as well as pigs (nos. 12, 13) kept in contact with infected animals, showed temperature elevation of 1.0–1.4° C. during 2–3 days and very slight clinical symptoms of respiratory disease (deep breathing, coughing) after 1–3 weeks. None died. There were no clinical changes in animals inoculated with the supernatant treated with oxytetracycline (nos. 16, 17) or chloroform (nos. 14, 15), or in the control pigs (nos. 18, 19).

*Gross lesions*

In our experiment no lesions were found in animals nos. 6 and 7 killed 1–2 weeks after inoculation. In animals inoculated with untreated supernatant of lung suspension and slaughtered 3–4 weeks after injection (nos. 8, 9) catarrhal pneumonia was observed. Similar lesions were found in pigs nos. 10 and 11 inoculated with *A. axanthum* broth culture and in the contact animals (nos. 12, 13), but in the former macroscopic lesions were slight. Animals inoculated with treated supernatant of lung suspension (nos. 14–17) did not show any alterations in the lung. All other organs examined were free from lesions.

*Histological picture*

In the lungs of animals (nos. 6–13) inoculated with untreated supernatant of lung suspension (nos. 6–9) and broth culture of *A. axanthum* (nos. 10, 11) and of pigs in contact with infected animals (nos. 12, 13), histological lesions of the interstitium (peribronchial lymphoid hyperplasia, peribronchiolitis, perivascularitis, intralobular interstitial pneumonia) and epithelium of bronchioli and alveoli (vacuolization, necrosis, desquamation of cells, proliferation of alveolar cells, alveolar macrophage reaction) were demonstrated (Table 1, Plates 1–3).

Lesions produced by pure culture of *A. axanthum* were not as severe as those in animals infected with untreated supernatant.

There were no lesions of the bronchiolar and alveolar wall in animals inoculated with oxytetracycline- and chloroform-treated supernatant of lung suspension, or in the control pigs. Very slight alterations of the interstitium were observed in

Table 1. *Histological lesions in the lung of pigs inoculated with untreated and treated lung suspensions and with Acholeplasma axanthum broth cultures intranasally*

Designation of animals	Lesions of the interstitium				Lesions of epithelium of bronchioli			Lesions of alveoli				
	Gross lesions in the lung	Peribronch. lymph. hyperplasia	Peri-bronchiolitis	Intra-lobul. interst. pneum.	Vacuolization	Necrosis	Desqua-mation	Prolifer-ation	Alveolar cell prolifer-ation	Alveolar macro-phage	Neutro-phyly gra-nulocya	Exsudate in alveoli
6	0	1	2	2	3	0	1	0	1	0	0	0
7	0	1	1	1	3	0	2	1	1	0	0	0
8	2	2	3	2	2	2	3	0	1	1	2	1
9	3	3	3	2	1	3	3	1	3	3	2	1
12	2	1	2	1	2	1	2	2	3	3	3	1
13	2	1	2	1	2	3	1	2	2	2	2	1
10	2	1	3	2	3	0	1	1	1	1	0	0
11	1	1	3	2	1	2	2	0	1	1	0	0
14	0	0	1	0	0	0	0	0	0	0	0	0
15	0	0	1	1	0	0	0	0	0	0	0	0
16	0	0	0	2	0	0	0	0	0	0	0	0
17	0	0	0	1	0	0	0	0	0	0	0	0
18	0	0	1	0	0	0	0	0	0	0	0	0
19	0	0	1	1	0	0	0	0	0	0	0	0

Notes: 0-3 = degree of severity and extension of lesions.

Table 2. Isolation of *Acholeplasma axanthum* from artificially infected pigs, and their serological response

Method of infection	Pig no.	<i>Acholeplasma</i> isolation		Time killed after inoc. (days)	<i>Acholeplasma</i> isolation after death from			Serum titres
		Before inoc.	After inoculation		Nasal cavity	Lung	Peribronch. lymph nodes	
Intranasal untreated lung suspension	6	—	—	7	—	—	—	—
	7	—	axan.	14	axan.	—	—	—
	8	—	axan.	21	axan.	axan.	axan.	1/4
	9	—	—	28	laid.	axan.	—	1/64
Contact animals	12	—	axan.	36	—	axan.	—	1/4
	13	—	—	36	—	axan.	—	1/16
Intranasal cloned broth culture of <i>A. axanthum</i>	10	laid.	—	41	—	axan.	—	1/32
	11	—	—	41	—	axan.	axan.	1/16
Intranasal chloroform-treated lung suspension	14	—	—	43	—	—	—	—
	15	—	laid.	29	laid.	—	—	—
Intranasal oxytetracycline-treated lung suspension	16	—	—	30	—	—	—	—
	17	—	—	43	—	—	—	—
Control animals	18	—	—	57	—	—	—	—
	19	—	—	57	laid.	—	—	—

axan. = *A. axanthum*; laid. = *A. laidlawii*.

the control animals and in pigs inoculated with oxytetracycline- and chloroform-treated material, but these lesions were not as severe as those in animals receiving material containing live *A. axanthum*.

#### *Isolation of Acholeplasma axanthum*

From the lung suspension used for inoculation only *A. axanthum* sp. was isolated. The examination of the suspension for the presence of other *Acholeplasma* and *Mycoplasma* species and for bacteria gave negative results. Virus isolation performed in swine kidney tissue cultures during four blind passages also gave negative result. Data of reisolations of *A. axanthum* strains from inoculated animals are shown in Table 2.

*Acholeplasma axanthum* could be isolated from most of the samples obtained from animals injected with untreated supernatant of the lung suspensions and broth cultures of *A. axanthum*, as well as from the contact pigs, whereas this species was not present in other animals. Some of the experimental animals were contaminated with *Acholeplasma laidlawii* strains identical with saprophytic *A. laidlawii* reference strains in the biochemical tests (Stipkovits, Schimmel & Varga, 1973). No other *Mycoplasma* and *Acholeplasma* species were detected, although the technique used was suitable for detecting all *Mycoplasma* and *Acholeplasma* species of swine (including *M. hyopneumoniae*).

#### *Serological examination*

A serological response was seen in animals inoculated with material containing live *A. axanthum* and in the contact animals, but not in the pigs nos. 14–19 (Table 2).

### DISCUSSION

The present paper demonstrates another successful isolation of *Acholeplasma axanthum* from the lung and peribronchial lymph nodes of a pig from a farm with catarrhal pneumonia (Stipkovits, Varga & Schimmel, 1973). By inoculation of animals with untreated supernatant of lung suspension containing *A. axanthum* mild clinical symptoms, definite gross lesions and histological changes of pneumonia were produced. Lesions of the same type were found in the lung of animals held in contact with pigs inoculated with untreated supernatant of lung suspension, confirming the ability of *A. axanthum* to spread by contact. Microscopic and macroscopic lesions which developed in the lung after infection of animals with broth culture of *A. axanthum* were similar to, but weaker than, those of contact animals. This observation could be explained by the possibility that the pathogenicity of *A. axanthum* might have decreased during the passages performed in artificial medium; thus it could produce slighter lesions only.

The negative results of inoculating animals with oxytetracycline- and chloroform-treated material are due to the killing effect of these substances on *Mycoplasma* and *Acholeplasma* species as demonstrated by Stipkovits, Schimmel, Molnár & Somos (1971) and Bögel, Berchthold, Brunner & Klinger (1962).

On the basis of clinical symptoms of respiratory disease, and pathological lesions in the lung of inoculated animals, the presence of *A. axanthum* in organs of pigs and their serological response to *A. axanthum* present in the material used for inoculation, a pathogenic role of this species might be supposed in the aetiology of pneumonia in swine. Specific pathogen free miniature pigs seem to be sensitive for the demonstration of such a pathogenic effect, although the animals are contaminated by saprophytic *A. laidlawii* species.

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## EXPLANATION OF PLATES

All sections are stained with H.E.

## PLATE 1

- Fig. 1. Peribronchiolar lymphocytic infiltration. Magnification,  $\times 54$ . Pig no. 10.  
Fig. 2. Perivascular lymphohistiocytic infiltration. Magnification,  $\times 346$ . Pig no. 11.  
Fig. 3. Thickening of the alveolar wall due to histiolymphocytic infiltration (intralobular interstitial pneumonia). Magnification,  $\times 139$ . Pig no. 11.

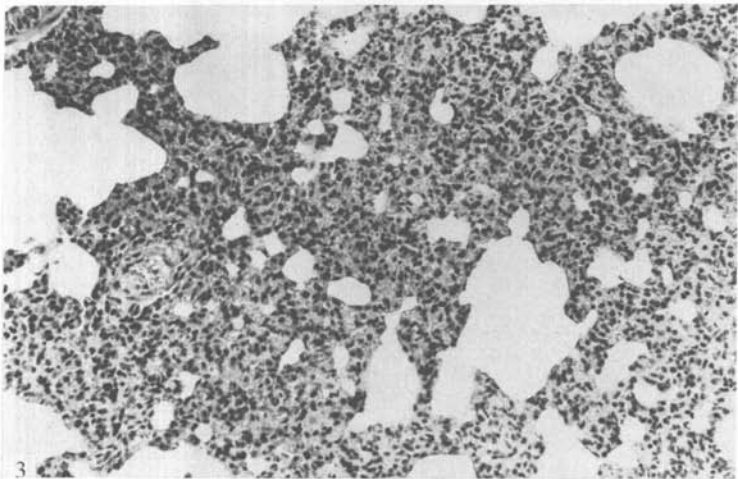
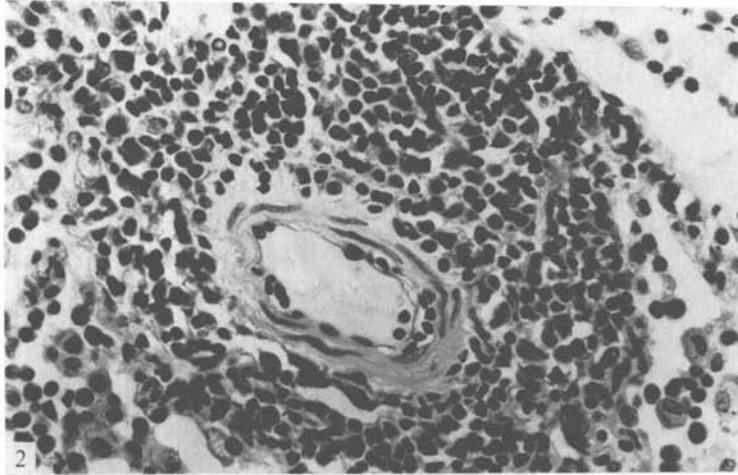
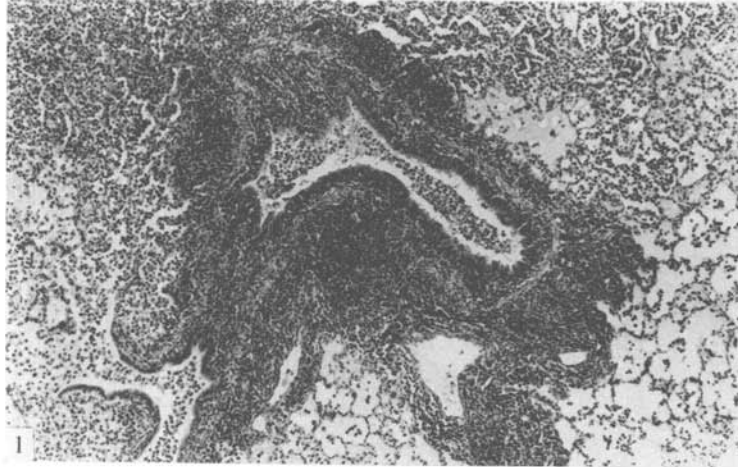
## PLATE 2

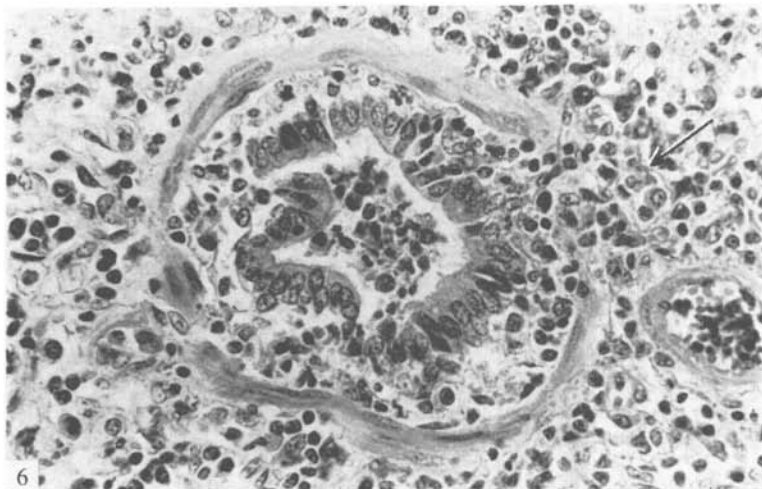
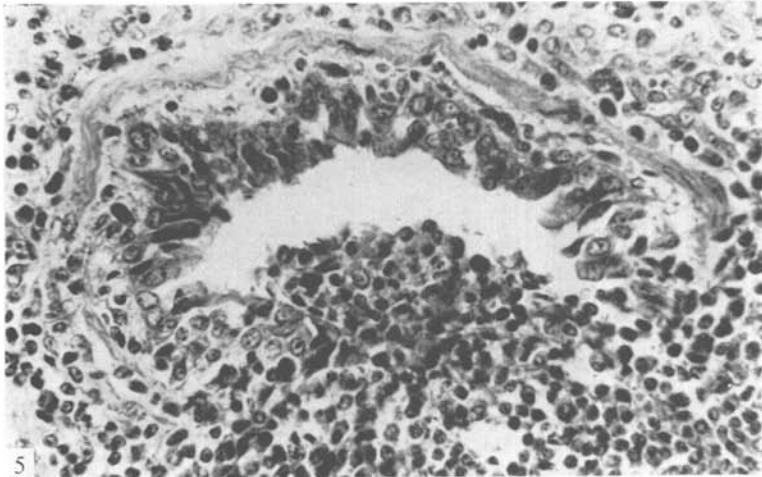
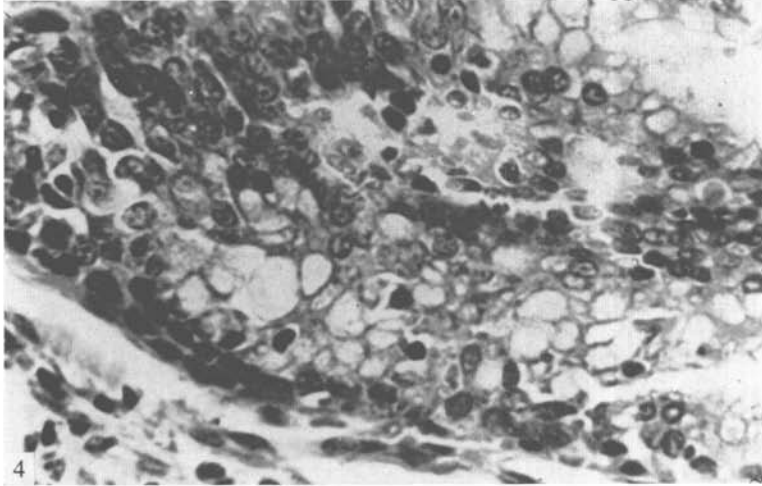
- Fig. 4. Vacuolization of bronchiolar epithelial cells. Magnification,  $\times 1350$ . Pig no. 10.  
Fig. 5. Severe infiltration in the propria of bronchiolar mucosa (see below). Epithelium lost definition over the infiltrated area. Magnification,  $\times 346$ . Pig no. 12.  
Fig. 6. Bronchiolar epithelium is desquamated, the propria is infiltrated with lymphocytes and histiocytes coming from the peribronchiolar connective tissue (arrow). Magnification,  $\times 346$ . Pig no. 7.

## PLATE 3

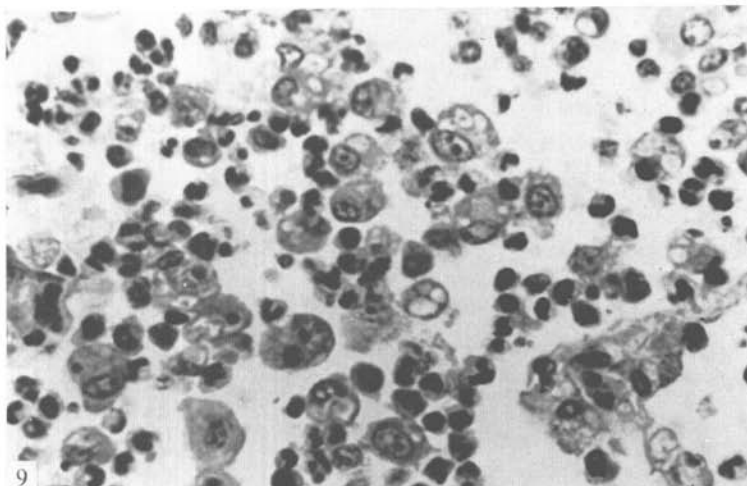
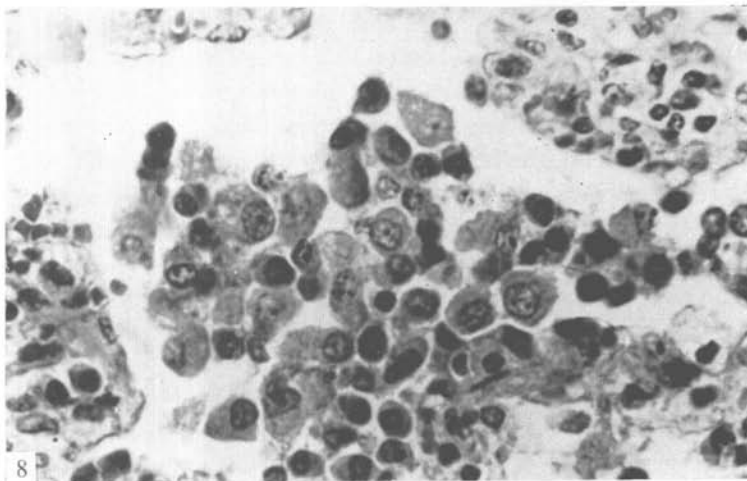
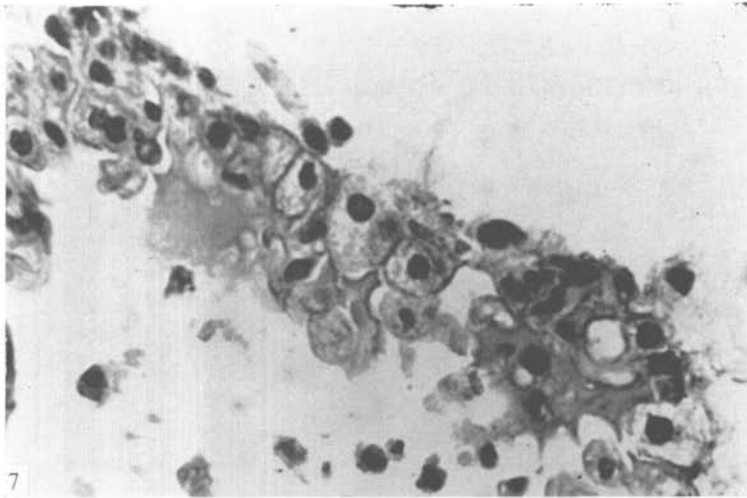
- Fig. 7. Vacuolized alveolar cells. Magnification,  $\times 1350$ . Pig no. 8.  
Fig. 8. Desquamated, non-vacuolized alveolar cells in the alveolar lumen. Magnification,  $\times 1350$ . Pig no. 9.  
Fig. 9. Alveolar macrophages, lymphocytes and neutrophil granulocytes in the alveolar lumen. Magnification,  $\times 1350$ . Pig no. 8.







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