Experimental scleroma

A histopathological study

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The concept of a bacterial aetiology for scleroma is now generally accepted. Convincing evidence testifying to the aetiological role of klebsiella rhinoscleromatis exists. This includes the regular incidence of this micro-organism in the tissues affected (Sinha *et al.*, 1969), immunofluorescence antibody studies (Hoffman *et al.*, 1963) and electron microscopic studies (Fisher and Dimling, 1962). The possibility of a virus-bacterial aetiology in scleroma has been postulated (Grewal, 1966). However, electron microscopic studies have shown no evidence of viral particles (Welsh *et al.*, 1963; Toppozada *et al.*, 1977). On the other hand, scanning electron microscopic studies (Gaafar and Harada, 1976) have revealed the presence of spores and hyphae of fungi in association with klebsiella rhinoscleromatis on the nasal mucous membrane.

Experimental animal inoculation by klebsiella rhinoscleromatis goes back to 1882 when Von Frisch reported the formation of small nodules at the site of injection in the nasal mucosa of mice with some histologic features of human scleroma. Other investigators later reported typical scleromatous lesions, either at the site of injection (Lasanga, 1925; Lobova, 1957) or as generalized septicaemia and/or granulomatous inflammation in the respiratory tract (Sercer, 1929; Steffen and Smith, 1961). Yet gaps still exist in establishing the role of klebsiella rhinoscleromatis, as seen from numerous reports of failure to satisfy Koch's postulates as regards experimental reproduction of the lesion in animals (Perkins, 1907), and the negative results of experimental infection in volunteers (De Simoni, 1899).

In view of this confusion in the actiological role of klebsiella rhinoscleromatis in producing scleroma, the present study aims to demonstrate the effect of experimental intranasal and intrasinal inoculation of klebsiella rhinoscleromatis in rabbits. Histopathological changes in the nose and paranasal sinuses as well as the lower respiratory tract are demonstrated.

Material and methods

(a) *Preparation of the bacterial inoculum.* The specimens used to prepare the bacterial inoculum were taken from a patient with rhinoscleroma in the granulomatous stage (Fig. 1). The specimens were placed in sterile Petri dishes and immediately processed. A strain of klebsiella having the

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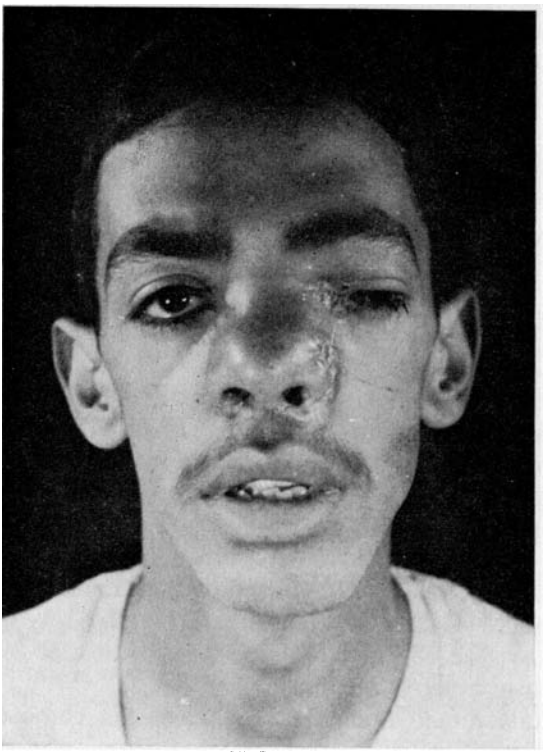


FIG. 1
Granulomatous stage of rhinoscleroma.

biochemical characters of the rhinoscleroma species and serologically belonging to type 3 was subcultured and purified on blood agar immediately after its isolation. Growth from the third subculture was suspended in sterile distilled water to form a suspension that contained 7×10^8 viable organisms per ml., as determined by the method of Miles and Mirsa (1938).

(b) *Routes of administration.* Eight healthy adult domestic rabbits (Oryctolagus cuniculus) were inoculated with the klebsiella suspension. Four of these were inoculated with 1 ml. intranasally, while in the other four animals 1 ml. of the suspension was injected into the maxillary sinus. Two healthy litter-mates served as controls.

(c) *Histopathological techniques.* The animals were killed at intervals varying from one to five months after the inoculation. The normal animals were killed after one and five months of starting the experiment. The animals were decapitated and the following tissues were immediately dissected out and immersed in 10 per cent formaldehyde solution: heads, larynges, tracheae, bronchi and parts of the lungs.

The heads were trimmed, then decalcified in 5 per cent nitric acid for 5 days. The nasal and paranasal cavities were then embedded in paraffin

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as one block. Sections of ten microns' thickness were cut by a non-continuous method for approximately forty sections per block (total of 400 sections). All the sections were stained with haematoxylin and eosin. The plane of sectioning used in all the animals was the frontal one. Pieces from other specimens were similarly processed and stained with haematoxylin and eosin. Histopathological changes in the tissues of the inoculated rabbits were evaluated after examining the normal controls.

Results

(a) *Nasal and paranasal findings:*

The anatomy of the nasal and paranasal cavities in the rabbit has been previously described (Kelemen, 1955). The nasal cavity is divided into two parts, one situated behind the other: the anterior (respiratory) and the posterior (olfactory) portion. The choanal region is situated at the boundary of the two portions. The respiratory portion shows one maxillo-turbinal and one nasoturbinal, while the posterior portion is filled by four

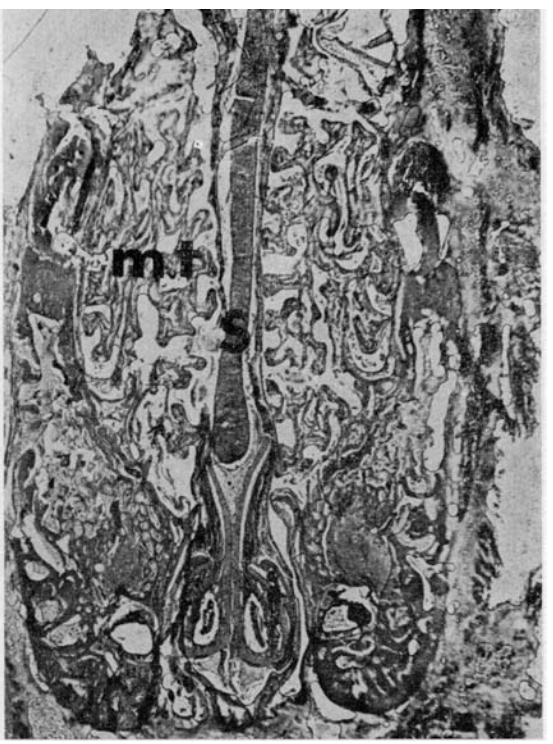


FIG. 2

Anterior part of the nasal cavity immediately behind the vestibulum. Note the catarrhal secretions among branches of the maxillo-turbinal (m.t.) and congestion of Jackobson's organ (j). Animal number 4, intranasal inoculation, 1½ months post-infection. Magnification about × 8.

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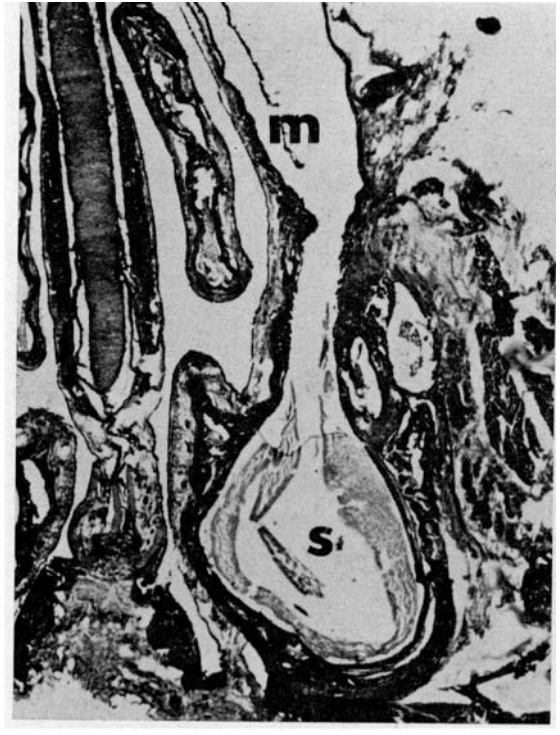


FIG. 3

Maxillary sinus (s) and marsupium (m) forming a common space. Note suppuration filling the sinus. Animal number 9, intra-sinal inoculation, 5 months post-infection. Magnification about × 8.

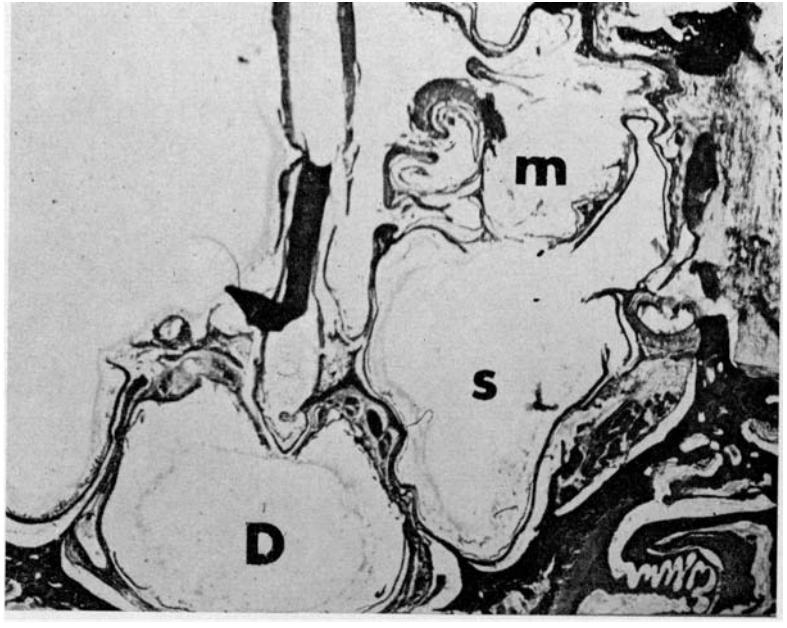


FIG. 4

Maxillary sinus (s) and marsupium (m) forming one space. The ostium is seen. Note the catarrhal secretions in the sinus and nasopharyngeal duct (D). Animal number 8, intra-sinal inoculation, 5 months post-infection. Magnification about × 8.

endoturbinals and four ectoturbinals. The rabbit has a wide maxillary sinus and no frontal sinus. The maxillary sinus is divided into anterior and posterior recesses which are in direct continuation with a lateral space called the marsupium. This latter is formed by the pneumatization of the nasoturbinal. Examination of the frontal series of sections (Figs. 2, 3 and 4) showed non-specific inflammatory reaction in the form of clear secretion, haemorrhagic exudate and suppuration. The lining mucous membrane (Fig. 5) showed thickening, metaplasia, vascular congestion and submucosal lymphocytic infiltration. In none of the animals did granulomatous reaction occur. The severity of pathological changes did not change with prolonging the period of survival nor did it differ with the method of inoculation.

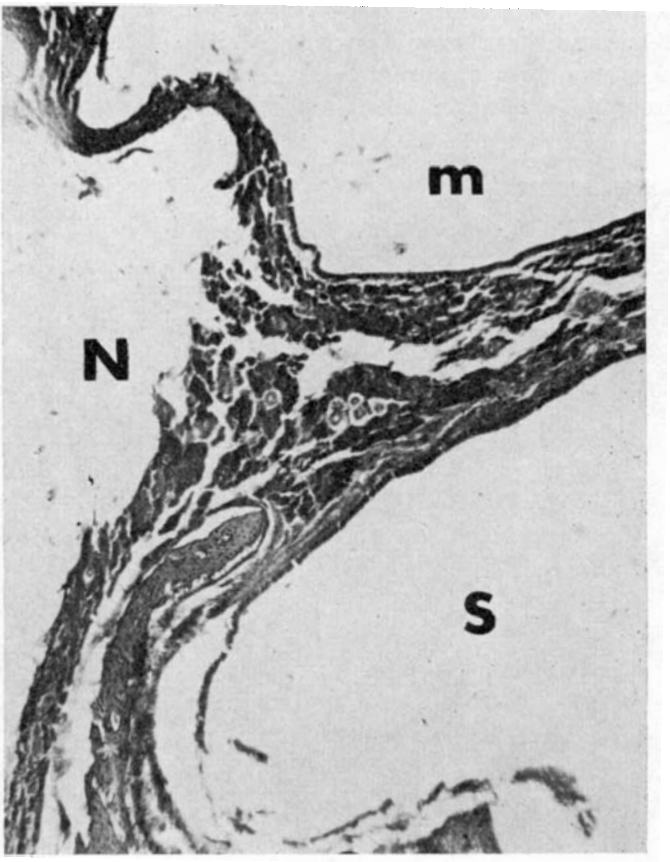


FIG. 5

Lining mucous membrane of the nasal cavity (N), marsupium (m), and sinus (s) after intra-sinal inoculation, 5 months post-infection. Note the thickening, metaplasia, congestion and lymphocytic infiltration. Magnification about × 50.

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(b) *Lower respiratory tract findings:*

Pathological changes following the injection of bacillus rhinoscleromatis were more obvious in the lower, than in the upper, respiratory tract. These changes were mostly seen in the peri-bronchial areas of the lungs. They became more marked with the passage of time, i.e. as the period increased between the inoculation and sacrifice of the animal. Naked eye appearances: the larynx, trachea and main bronchi showed no apparent gross changes. There was a marked pleural effusion, which was haemorrhagic in nature in those animals sacrified late in the experiment.

The lungs showed no significant changes in the early stages. At a later stage, some dispersed foci of bronchopneumonia appeared as irregularly consolidated areas, dark red in colour with a liver-like appearance. These broncho-pneumonic foci alternated with areas of normal collapsed lung tissue. As the survival time of the rabbits became longer, these foci of bronchopneumonia became wider, and more confluent. The pleura covering the lung surface showed evidence of pleurisy, looking dull opaque and losing the normal translucent shiny appearance.

Microscopic appearances: Occasionally, areas of focal infiltration by

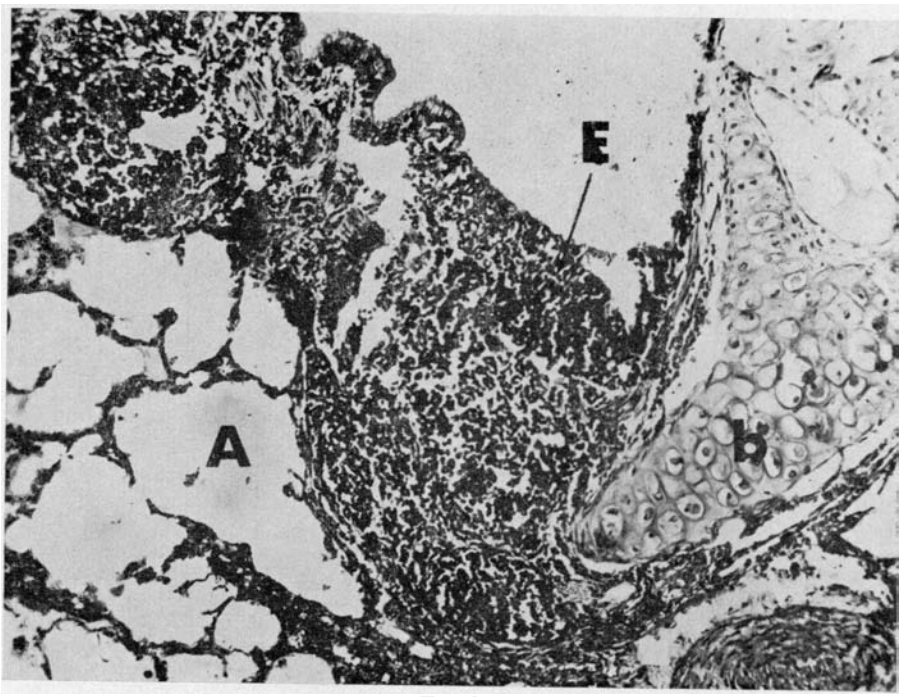


FIG. 6

Mononuclear granulomatous inflammatory exudate (E) surrounding a main bronchus (b). Rabbit number 1, 1 month after intra-nasal inoculation. A, alveoli. Magnification about × 300.

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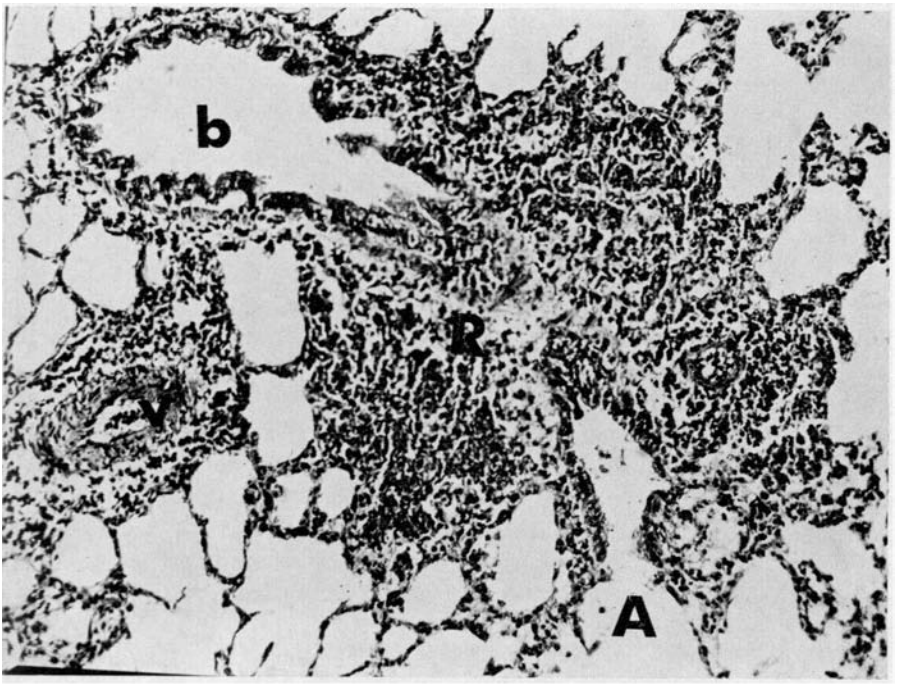


FIG. 7

Peribronchial (b) and perivascular mononuclear granulomatous inflammatory reaction (R). Rabbit number 3, 1½ months after intra-sinal inoculation. A, alveoli. Magnification about × 300.

chronic mononuclear inflammatory reaction were seen to be surrounding the main bronchi (Fig. 6). In the lungs, consolidated areas showed congestion and some focal areas of haemorrhage inside the alveoli. A granulomatous non-caseous inflammatory reaction was seen. This reaction was mainly concentrated in the perivascular and peribronchial areas (Fig. 7). The mature portion of the granuloma appeared nearer to the vessel wall while the less intense immature portion of the granuloma was towards the periphery.

This granulomatous reaction was made up of mononuclear cellular infiltration showing lymphocytes, eosinophils, histiocytes and many vacuolated foamy 'Mikulicz-like cells' (Fig. 8). There were many plasma cells, some of which were changing into mott cells containing 'Russel's bodies'. The foci of this granulomatous reaction became more confluent and wider in area as the survival time of the rabbits increased until a diffuse pneumonic reaction involving the whole lung was noticed in late cases (Fig. 9).

Discussion

The present work has failed to demonstrate the specific granulomatous reaction of rhinoscleroma at the sites of inoculation in the nose and

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maxillary sinus of rabbits. This negative finding was previously demonstrated by other investigators in human volunteers (De Simoni, 1899) and in experimental animals (Steffen and Smith, 1961; Lum, 1971). The nonspecific histopathological changes in the nose and sinuses can be attributed to the reaction of tissues to the injected solution.

On the contrary, the lower respiratory tract has shown a typical granulomatous reaction of scleroma. This reaction was located mainly in the peribronchial and perivascular areas. The mature part of the granuloma was the part nearer to the vessel wall which suggests a descending infection along the bronchial tree. These findings are in accordance with the findings of Steffen and Smith (1961) who produced experimental pneumonia with granulomatous process in the lungs of mice after intranasal inoculation. This controversy on the pathogenicity of klebsiella rhinoscleromatis in the upper and lower respiratory tracts may be attributed to some of the morphological characters of the bacillus itself. In electron microscopic studies (Gonzalez-Angelo *et al.*, 1964; Toppozada *et al.*, 1977) it was found that the organisms were different in culture and inside Mikulicz cells. In culture, the slime layer around the bacilli is very thin, with practically no fimbriae. Inside Mikulicz cells, the bacilli have a

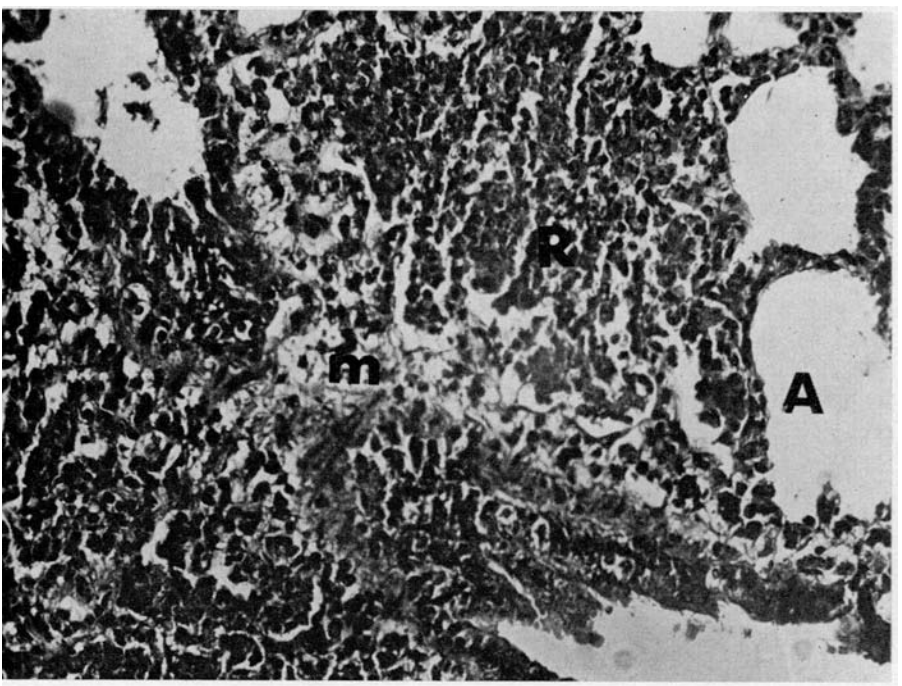


FIG. 8

Mononuclear granulomatous reaction (R) showing lymphocytes, eosinophils, histiocytes and vacuolated foamy cells (m). A, alveoli.
Magnification about × 700.

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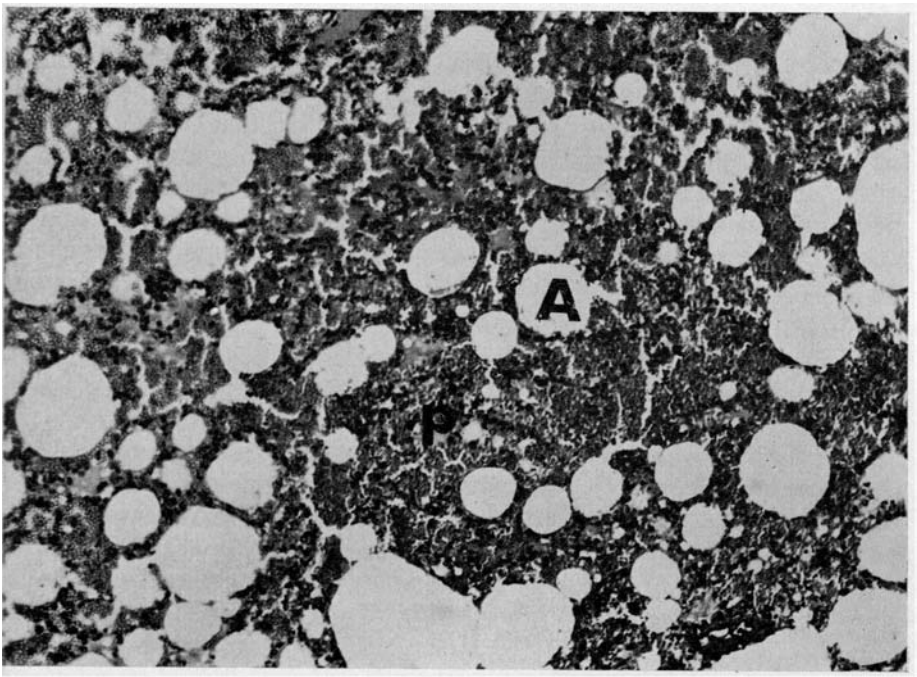


FIG. 9

Diffuse pneumonic reaction (p) of the whole lung (A). Rabbit number 9, 5 months after intrasinal inoculation.
Magnification about × 300.

thick slime layer and many long fimbriae. These fimbriae act as organs of attachement since most fimbriated strains have the power to adhere to animal and fungal cells (Duguid, 1959). These differences may explain the absence of scleromatous lesions in the nose and sinuses of experimental animals since the slime layer of the bacilli may develop only in the lower respiratory passages as a secretion from the bacillus itself or from the surrounding alveolar secretion. The slime layer was found to be made up of mucopolysaccharides (Gonzalez-Angelo *et al.*, 1964). A chemical study of the nasal and alveolar secretions is necessary to document such a hypothesis. Other factors which may help in the development of scleromatous lesions in the lower respiratory tract include pH of the alveoli, oxygen saturation, ciliary action, etc.

Contrary to experimental animals, rhinoscleroma in humans seems to be rare in the lower respiratory tract. Holinger *et al.* (1977) found tracheal scleroma in 12 per cent and bronchial scleroma in 7 per cent. In all their cases, involvement of the trachea and bronchi was secondary to scleroma of the nose. This discrepancy in the incidence of upper versus lower respiratory involvement in man and animals deserves further investigation.

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Summary

Klebsilla rhinoscleromatis suspension was experimentally inoculated into the nose and maxillary sinus of eight adult healthy rabbits. Two normal litter-mates served as controls. The animals were killed at varying intervals up to five months following the inoculation. Histopathological examination of the upper and lower respiratory tracts revealed a consistent pulmonary peribronchial and perivascular granulomatous reaction with a Mikulic cell-like appearance. The reaction was present in all the inoculated rabbits and increased in severity as the survival period was prolonged. The significance of these findings in the aetiopathogenesis of scleroma and to scleroma in humans is discussed.

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