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HERPES NODULES IN THE LUNG OF THE AFRICAN ELEPHANT [*LOXODONTA AFRICANA* (BLUMENBACH, 1797)]

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ABSTRACT

McCULLY, R. M., BASSON, P. A., PIENAAR, J. G., ERASMUS, B. J. & YOUNG, E., 1971. Herpes nodules in the lung of the African elephant [*Loxodonta africana* (Blumenbach, 1797)]. *Onderstepoort J. vet. Res.* Vol 38 (4), 225–236 (1971).

Lymphoid nodules associated with Cowdry Type A intranuclear inclusions in epithelial and syncytial cells were found in the lungs of 74% of 50 African elephants in the Kruger National Park. Subsequent studies proved these were caused by a herpes virus (Erasmus, McCully, Pienaar, Young, Pieterse & Els, 1971). The disease appears to be subclinical or latent. This virus, in common with other herpes viruses, might be more pathogenic in some other host. The pathogenesis of the lymphoid nodules and the various stages of their formation are given and the detailed characteristics are illustrated.

INTRODUCTION

Post mortem examination of elephants killed during a cropping programme in the Kruger National Park revealed that many of them contained nodules of variable size and number in their lungs. A herpes virus was suspected when Cowdry Type A intranuclear inclusions were found microscopically in the epithelial cells of these nodules. This finding prompted further studies that led to the demonstration by electron microscopy (EM) of virus particles associated with the inclusions (McCully, Basson, Pienaar, Erasmus, Young & Pieterse, 1969; Basson, McCully, Kruger, Van Niekerk, Young, De Vos, Keep & Ebedes, 1971), and to the isolation and characterization of the virus. It was concluded that it was a member of the herpesvirus group (Erasmus, McCully, Pienaar, Young, Pieterse & Els, 1971; Basson, McCully, De Vos, Young & Kruger, 1971). This report is primarily concerned with the histopathology and pathogenesis of these lung nodules.

MATERIALS AND METHODS

Fifty elephants selected at random were shot, sometimes after initial immobilization with scoline. Their lungs were superficially examined, palpated and then cut into many slices with a sharp knife. Superficial examination was very difficult due to the absence of a true pleural cavity and the presence of a thick, loose layer of endothoracic fascia in the adult elephant (Evans, 1910; Hill, 1953). Close inspection, however, revealed a small area over the diaphragmatic lobes where the parietal pleura was unattached to the visceral layer. This formed a very shallow potential pleural cavity. Blunt dissection from this point cleared the visceral pleura fairly easily from the loose areolar tissue of the parietal layer. By using this technique the smooth outer surface of the lung, very similar to that of other animals, was exposed for examination.

Some of the lesions encountered were collected either in 10% buffered formalin or Zenker's solution. From these specimens appropriate blocks were cut and embedded in paraffin wax. Sections of 3 to 6 μ thickness were prepared with a sliding microtome and routinely stained with haematoxylin and eosin (HE).

Material for electron microscopy was collected from the lung nodules of two elephants. This was fixed in 4% glutaraldehyde in Millonig's buffer (Millonig, 1961), post-fixed in 2% osmium tetroxide, dehydrated and embedded in Araldite* (Luft, 1961). Thin sections for electron microscopy were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963).

Fresh material from the lymphoid nodules of the lungs of 10 elephants was collected for virological studies (Erasmus *et al.*, 1971).

RESULTS

Macroscopic findings

Out of a total of 50 elephants, 74% contained from 1 to 6 rounded, greyish-white lymphoid nodules, some with two or three lobes, in the lungs [Fig. 1 (1)]. They varied in diameter from 3 to 30 mm, with an average of about 10 mm. They were easily detectable on the cut surface but were rarely noticeable as raised areas on the pleural surface of the lungs from which the adherent parietal pleura had been removed. The elevated, rounded lesions had either a fairly firm or a somewhat spongy consistency. They were enveloped either by lung tissue or by thin layers of fibrous tissue, which anatomically appeared to be interlobular septa. Many of the nodules could easily be enucleated from the surrounding tissue. On cut section, the more compact nodules were found to consist of a fairly solid mass of greyish-white lymphoid tissue, but those that were spongy contained cavities varying in size and number and surrounded by an irregular zone of lymphoid tissue. The cavities were either filled with air, a straw-coloured fluid or a gelatinous semi-solid material.

Solid nodules which protruded into the lumen of the bronchi [Fig. 1 (2)] were occasionally encountered. Their diameter varied from that of a pinhead to about 10 mm.

The solid nodules were more frequently noticed in young and the spongy ones in old elephants. Some of the adults, however, had both types. Areas of associated pneumonia were never found, but one case was com-

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plicated by foci of mycotic pneumonia. The bronchial and mediastinal lymph nodes sometimes appeared mildly hyperplastic.

Microscopic findings

Both the solid and the spongy nodules consisted mainly of areas of lymphoid proliferation in which some of the secondary lymphoid nodules with germinal centres were separated from one another by very narrow epithelial-lined spaces [Fig. 3 (13)]. In the spongy nodules some of these spaces had developed into large confluent cavities [Fig. 6 (33)]. The solid lesions appeared to be more hyperplastic while the spongy nodules were more often regressive in nature. Cowdry Type A intranuclear inclusions were frequently encountered in many of the epithelial cells of both lesions [Fig. 3 (15) and 7 (39)].

The two earliest changes which seemed to lead to the formation of these lung nodules were metaplasia and hyperplasia of the alveolar lining cells and either an almost simultaneous or subsequent lymphoid proliferation [Fig. 1 (3, 4 and 5)]. The alveolar lining cells became cuboidal and lymphocytes appeared within the alveolar septa. The primary sites of lymphocytic proliferation seemed to vary and were encountered in the alveolar septa [Fig. 2 (8)] around the alveolar ducts and around the bronchioli [Fig. 1 (6) and 2 (7)]. Remnants of smooth muscle were observed in some of them. Proliferation frequently started along the interlobular septa [Fig. 2 (9, 10 and 11)] from where it proceeded into the lung lobuli, thus involving the septa around the alveolar ducts and between alveoli. Lymphoid nodules, however, were also found in the walls of some of the bronchi. They differed from the others because there were no epithelial cells entrapped and no viral inclusions present. These bronchial nodules, which are evidently enlarged, are thought to represent a normal distribution of lymphoid tissue in the bronchial tree of the elephant.

The progressive development of these lesions resulted in the formation of the pulmonary nodules, which were macroscopically visible. Lymphoid nodules with distinct germinal centres [Fig. 2 (12)] developed and large lymphocytic macrophages, which gave the nodule a typical "starry sky" appearance, were frequently noticed. The pulmonary lobuli were eventually either partially or completely transformed into areas of lymphoid tissue. Whenever this transformation was complete, interlobular septa entirely surrounded the lesion. This phenomenon at times gave the false impression of encapsulation. The entire process frequently reduced the lumens of the alveoli and alveolar ducts to very narrow spaces [Fig. 3 (13)], but some larger cavities were also formed [Fig. 3 (14) and (17)]. Usually these spaces only partially surrounded some of the lymph nodules.

The epithelial cells lining the spaces between the lymphoid nodules varied in shape from cuboidal to columnar [Fig. 6 (35)] and their cytoplasm was either partially or entirely vacuolated or fairly mildly eosinophilic and homogenous. Some of these cells were evidently either hypertrophic or metaplastic alveolar lining cells [Fig. 3 (14, 15 and 17)] and epithelial cells of the alveolar ducts [Fig. 3 (16)]. They contained intranuclear inclusions, which had slightly variable morphological and tinctorial features. With HE the early stages of the inclusions were usually either elongated or somewhat circular and purplish-blue in colour with a few fine peripheral strands of chromatoid material. The later stages were more rounded, purplish-red in colour and each one was surrounded by a distinct halo. Although some smaller respiratory bronchioli could also have been present in some of the nodules, unequivocal evidence of inclusions in the bronchiolar epithelial cells or in those lower down the bronchial tree was never obtained.

Some epithelial cells in the nodules proliferated loosely [Fig. 3 (18)] while others formed a solid and medullary pattern, frequently obliterating the enclosed cavities entirely [Fig. 4 (19 and 20)]. Other cells proliferated in a frondlike manner along a common septal core [Fig. 4 (22, 23 and 24)]. Syncytia and multinucleated giant cells containing inclusions were commonly seen [Fig. 4 (21) and 5 (25, 26 and 27)]. With increasing age eosinophilic intracytoplasmic bodies of variable size appeared in these cells [Fig. 5 (28, 29 and 30)]. Some of the cells became hyalinized and eventually calcified [Fig. 6 (31 and 32)]. Scattered Russel body plasmocytes were at times noticeable in these zones of epithelial proliferation. Individual or small groups of apparently dislodged cells were occasionally encountered within the lymphoid nodules.

The enclosed spaces between the epithelial cells were either obliterated or enlarged to form cavities [Fig. 6 (33)]. They were either empty or filled with proteinaceous fluid and were more frequently, but not invariably, encountered in the regressive nodules, which appeared spongy macroscopically. The patterns of epithelial hyperplasia around these cavities were as described above, but polypoid and papillary proliferations were also present [Fig. 6 (34)]. Many epithelial cells, giant cells or syncytia became detached and were suspended in the proteinaceous fluid [Fig. 6 (36) and 7 (37)]. Some remained viable and had inclusions [Fig. 7 (37 and 38)] but others were degenerative and necrotic. Small numbers of polymorphonuclear cells were occasionally noticed within the cavities.

Regression and ageing of the nodules were characterized by the epithelial mineralization described above, lymphoid atrophy and thickening and hyalinization of some of the alveolar walls and interlobular septa [Fig. 7 (40, 41 and 42)]. Scattered lymphocytes were still present in these areas and occasionally the epithelial lining became either low cuboidal or squamous [Fig. 7 (39)].

Electron microscopy

Examination by electron microscopy showed that the intranuclear inclusions in the epithelial and giant cells [Fig. 8 (43)] consisted of a mass of finely granular material with which numerous viral particles were associated [Fig. 8 (45)]. The viral particles were either scattered singly within and around the granular mass or appeared in clusters [Fig. 8 (44 and 45)]. Morphologically these viral particles were consistent with the herpesvirus group and a detailed description of them is given elsewhere (Erasmus *et al.*, 1971).

Discussion

A viral etiology was suggested by the presence of typical Cowdry Type A intranuclear inclusions within the epithelial cells of the lymphoid nodules of the lung (McCully *et al.*, 1969). The subsequent demonstration of virus particles associated with the inclusions by EM and the isolation and characterization of this virus proved that it is a member of the herpesvirus group (Erasmus *et al.*, 1971). It seems quite probable that the virus is contracted by inhalation though the possibility of other routes of infection is not excluded.

The spectrum of lesions produced by viruses of various types ranges from hyperplasia and neoplasia on the one end to necrosis on the other. Lesions caused by

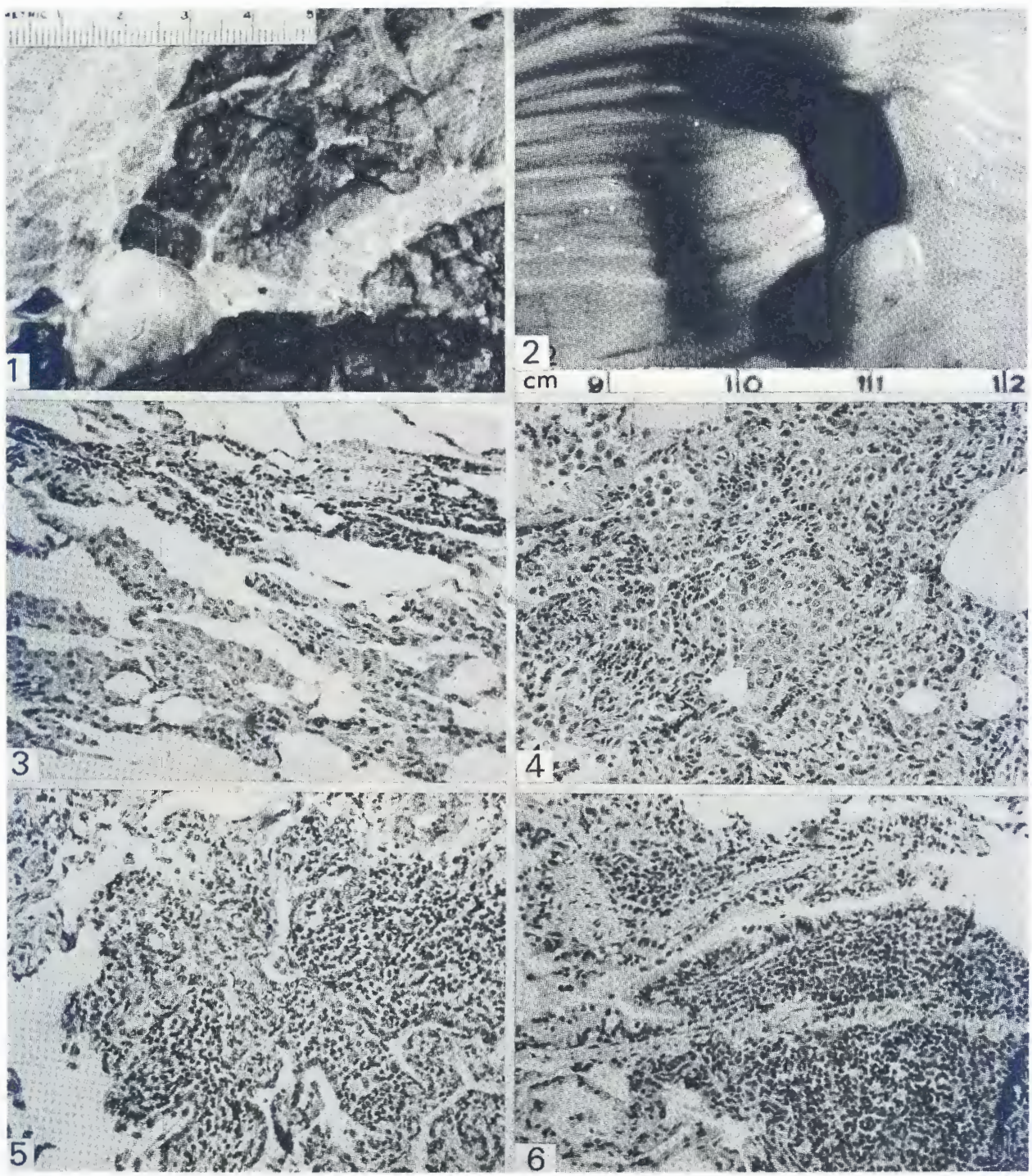

FIG. 1 1. Lymphoid nodule within the lung tissue as seen macroscopically on section 2. Small lymphoid nodule at the opening of a small bronchus 3. Hyperplasia and metaplasia of the alveolar lining cells and concomitant mild lymphocytic infiltration of the alveolar septa (top). HE × 150 4. Alveoli containing several epithelial cells. HE × 150 5. Alveolar septa considerably thickened by the presence of large numbers of lymphocytes. Metaplasia of alveolar lining cells is prominent. HE × 150 6. An alveolar duct branching from a small bronchiole (left). Lymphocytes are present in the walls of both, but are more numerous in the duct. HE × 150

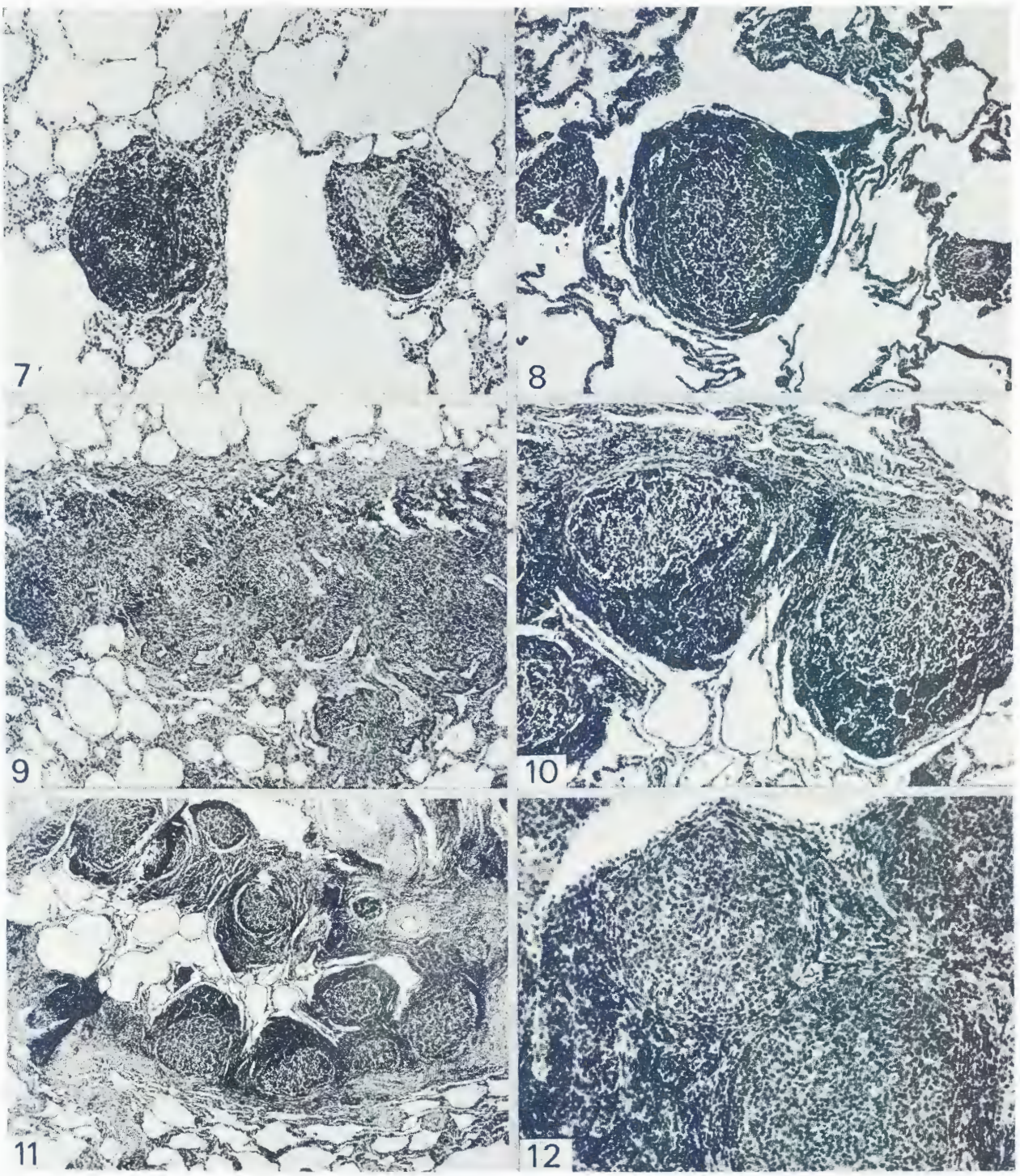

FIG. 2 7. Small lymphoid nodules adjacent to an alveolar duct and small artery. HE × 75 8. A small lymphoid nodule in an alveolar wall close to an alveolar duct. HE × 75 9. Lymphoid proliferation along an interlobular septum with progression into the adjacent alveolar septa. Metaplasia of the affected alveolar epithelium is a prominent feature. HE × 45 10. Lymphoid nodules along an interlobular septum. HE × 75 11. Lymphoid proliferation along interlobular septa. HE × 30 12. An inner portion of a large lymphoid nodule with distinct reaction centres and an inner single row of vacuolated epithelium (top). HE × 150

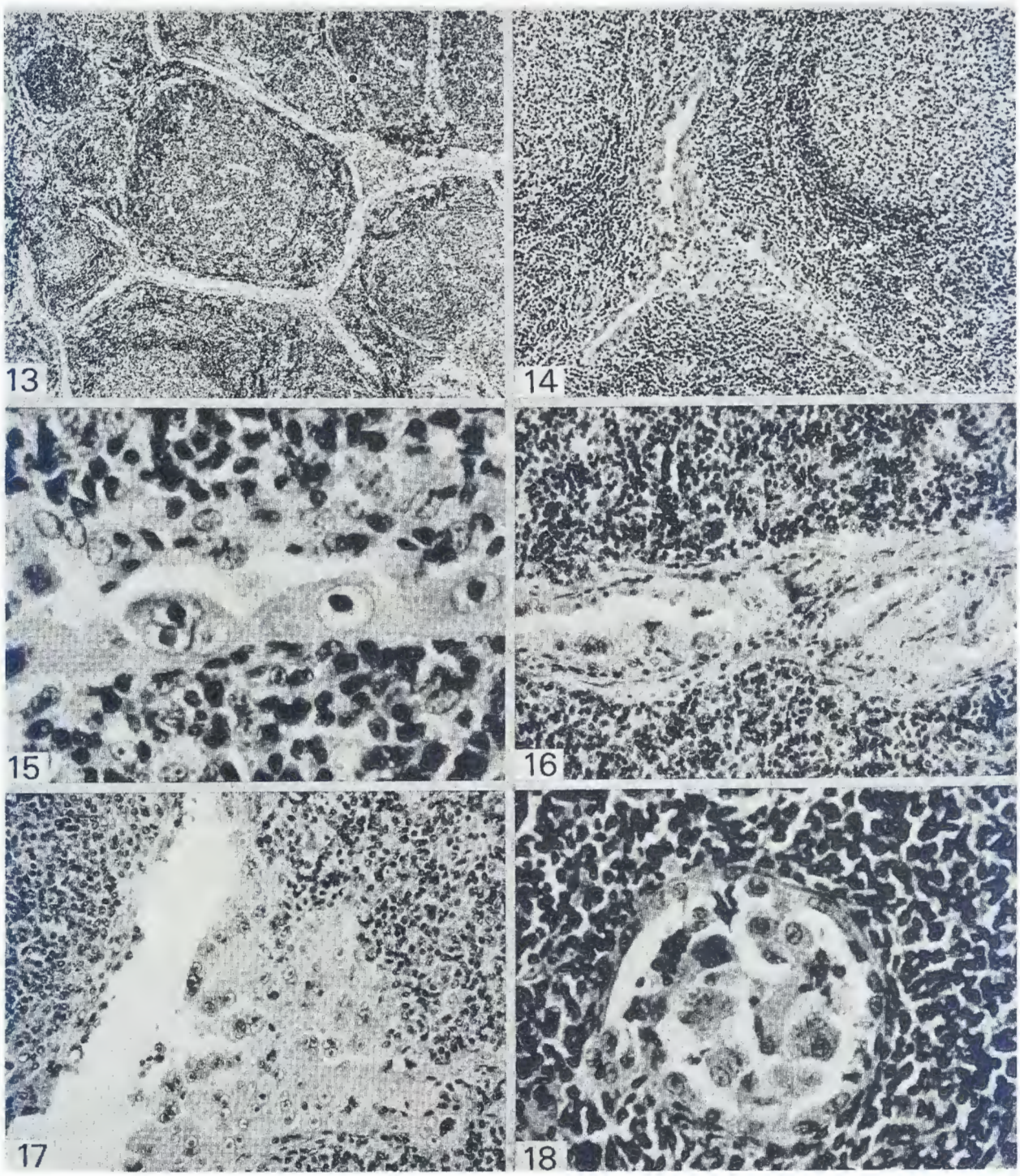

FIG. 3 13. An inner portion of a nodule. Several secondary nodules with reaction centres partially surrounded by narrow epithelial-lined spaces. HE × 75 14. Hyperplastic and metaplastic alveolar lining cells within a nodule, with a small number of round cells within the alveolus. HE × 110 15. Intranuclear inclusions in the lower row of alveolar cells within a nodule. The alveolar space is compressed to a narrow cavity. HE × 500 16. Intranuclear inclusions in some of the epithelial cells of an alveolar duct within a nodule. The very thin but distinct muscular layer of the duct is evident. HE × 200 17. Many inclusions in the metaplastic and hyperplastic alveolar lining cells of an enclosed alveolus. HE × 200 18. Transverse section of a group of rather loosely arranged epithelial cells. HE × 150

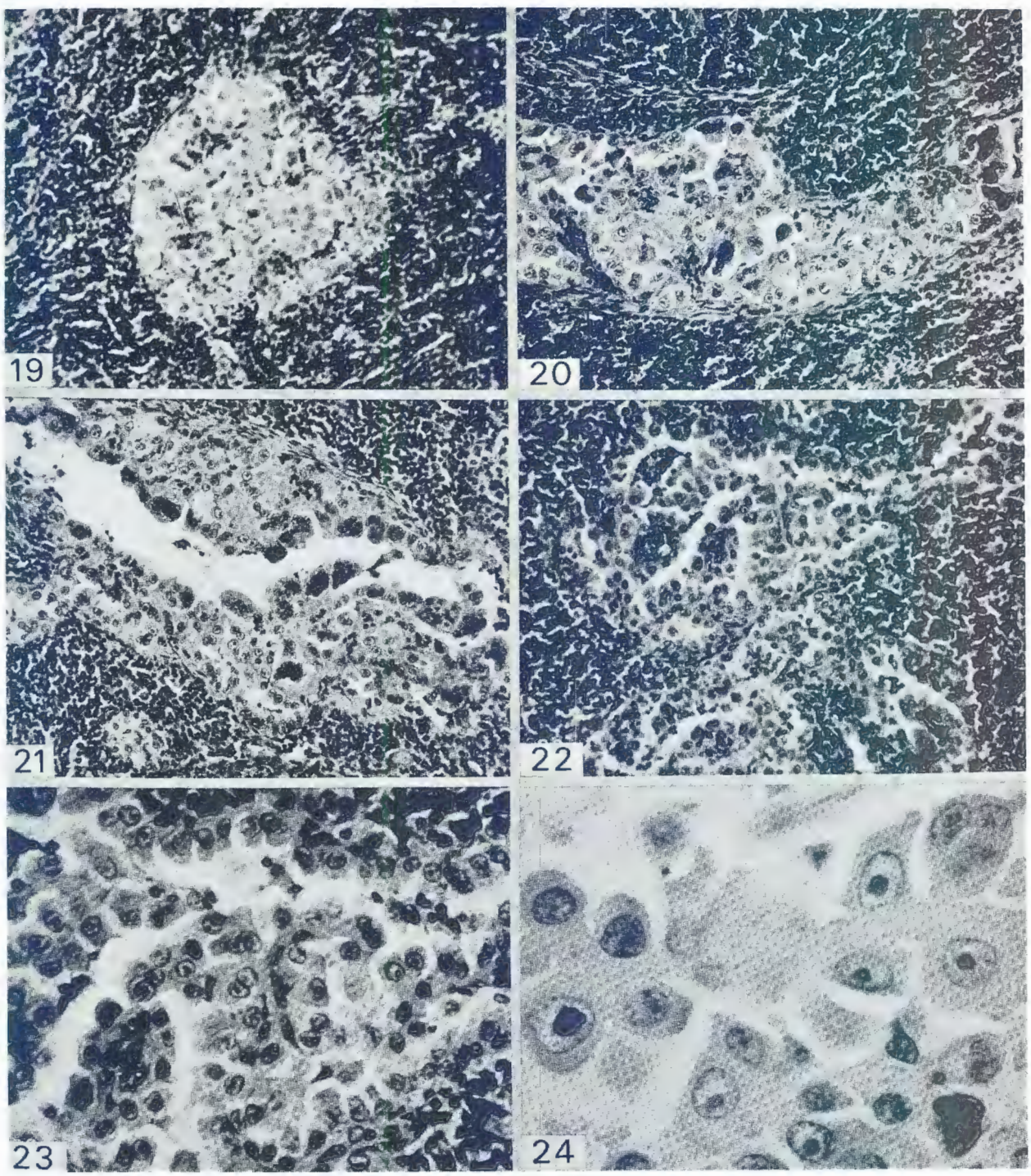

FIG. 4 19. Transverse section through a core of epithelial cells in a nodule which is growing in a solid or medullary pattern. HE × 150 20. Longitudinal section through a cord of epithelial cells amidst lymphoid proliferation. Intranuclear inclusions are also present. HE × 150 21. Formation of many giant cells in the proliferated, inclusion-bearing epithelium of an alveolar duct. HE × 150 22. Frond-like pattern of epithelial proliferation and metaplasia. HE × 150 23. Higher magnification of an area from the previous photomicrograph (22). The cells are either cuboidal or low columnar and some intranuclear inclusions can be seen. HE × 320 24. High magnification of epithelium proliferating in a frond-like pattern and a few Type A intranuclear inclusions. HE × 750

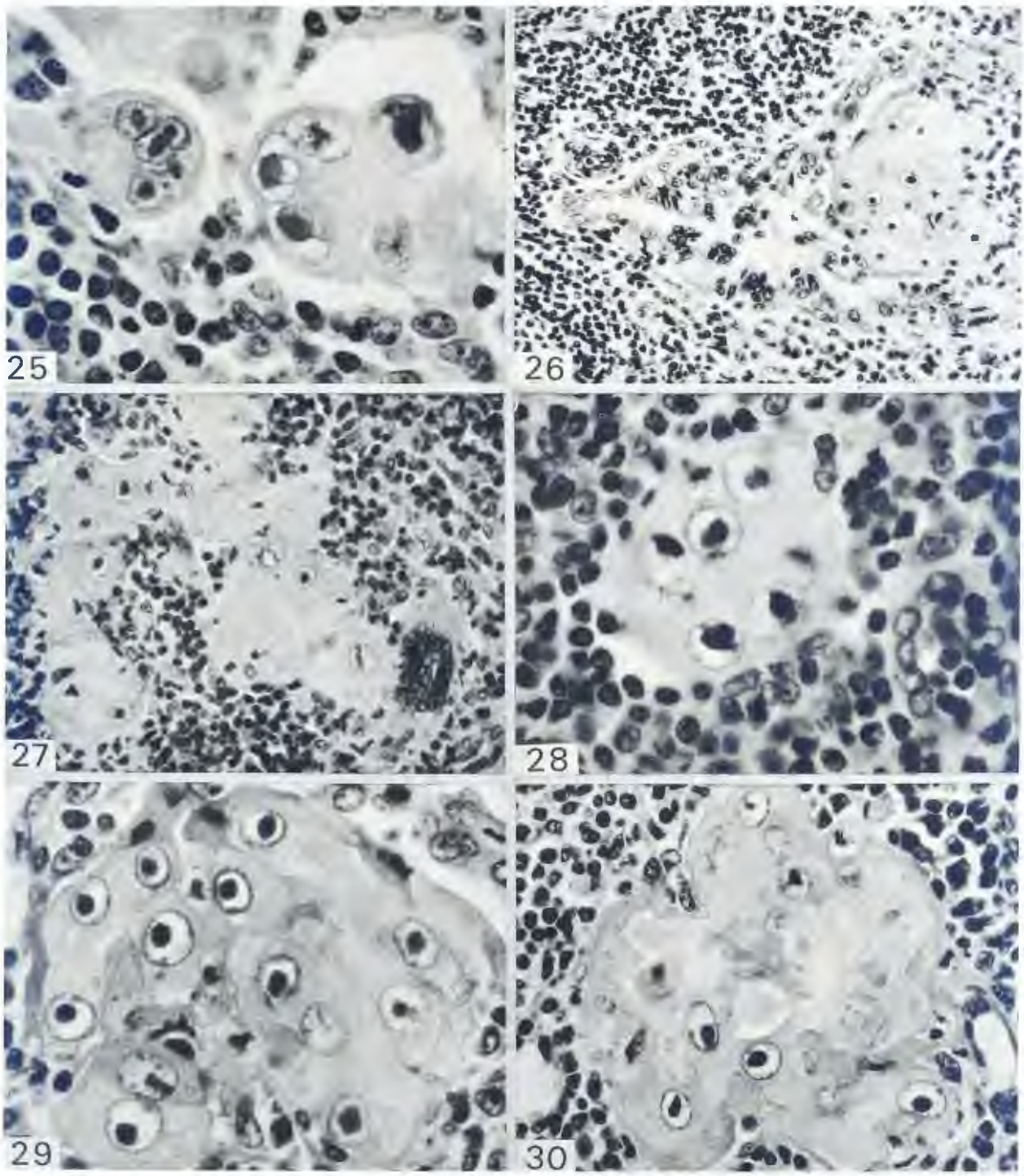

FIG. 5 25. Two giant cells with inclusions in every nucleus. HE × 750 26. Syncytial giant cell formed in the wall of an alveolar duct. HE × 200 27. A large irregular syncytial mass of epithelial cells containing intranuclear inclusions. A large group of densely arranged nuclei is noticeable in the lower right corner. HE × 75 28. Giant cell with inclusion bodies and a few small eosinophilic intracytoplasmic globules. HE × 750 29. Large syncytium with intranuclear inclusions and deeply eosinophilic intracytoplasmic material. HE × 700 30. Large inclusion-bearing syncytium with larger and more numerous eosinophilic material. HE × 500

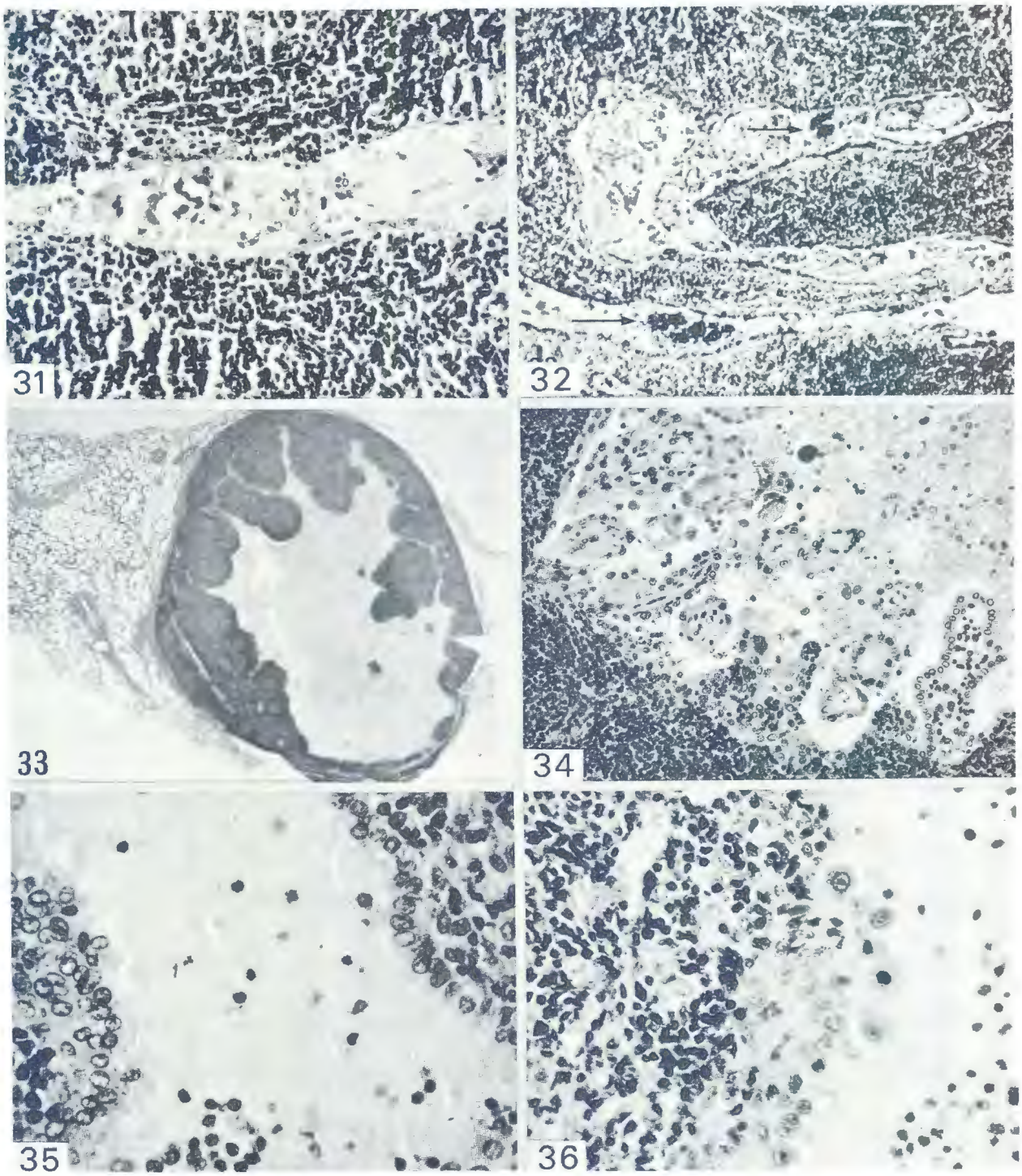

FIG. 6 31. Degenerating and necrotic syncytial cells in a metaplastic alveolus. Only a small number of nuclei is present in the masses of eosinophilic cytoplasm. HE × 200 32. Hyalinization and calcification of epithelial cells. HE × 115 33. Lymphoid nodule with a cavity filled with eosinophilic proteinaceous fluid containing some epithelial cells. HE × 5 34. Papillary projections into a fluid-filled cavity in one of the nodules. HE × 150 35. Cavity of a nodule (illustrated in 33), lined in some areas by either cuboidal or low columnar epithelium. HE × 320 36. Some epithelial cells which are only attached by narrow pedicles to the wall of the cavity in a nodule. HE × 300

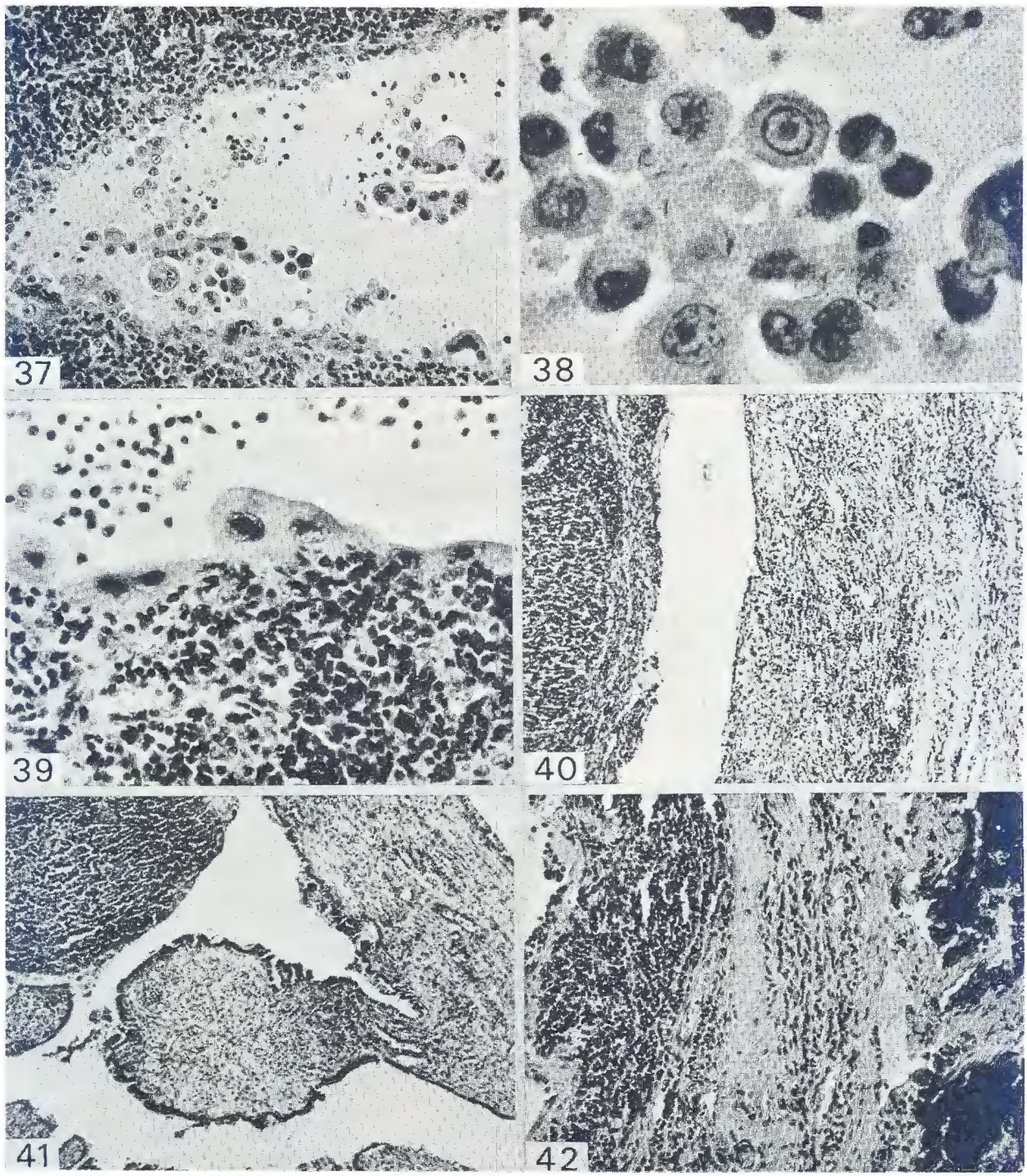

FIG. 7 37. Detached epithelial cells and epithelial cell syncytia in a fluid-filled cavity of a nodule. Intranuclear inclusions are also present. HE × 150 38. A cavity in a nodule with viable, degenerating and necrotic epithelial cells. One cell has a distinct intranuclear inclusion. HE × 750 39. Cavity in a nodule lined by inclusion-bearing, flat to low cuboidal epithelium. HE × 300 40. Portion of a regressive nodule as indicated by the paucity of lymphocytes and mild fibrosis. HE × 75 41. Portion of a regressive spongy nodule with more advanced fibrosis in the thickened alveolar septa. HE × 75 42. Partial calcification (left) and some encapsulation in a regressive spongy nodule. HE × 75

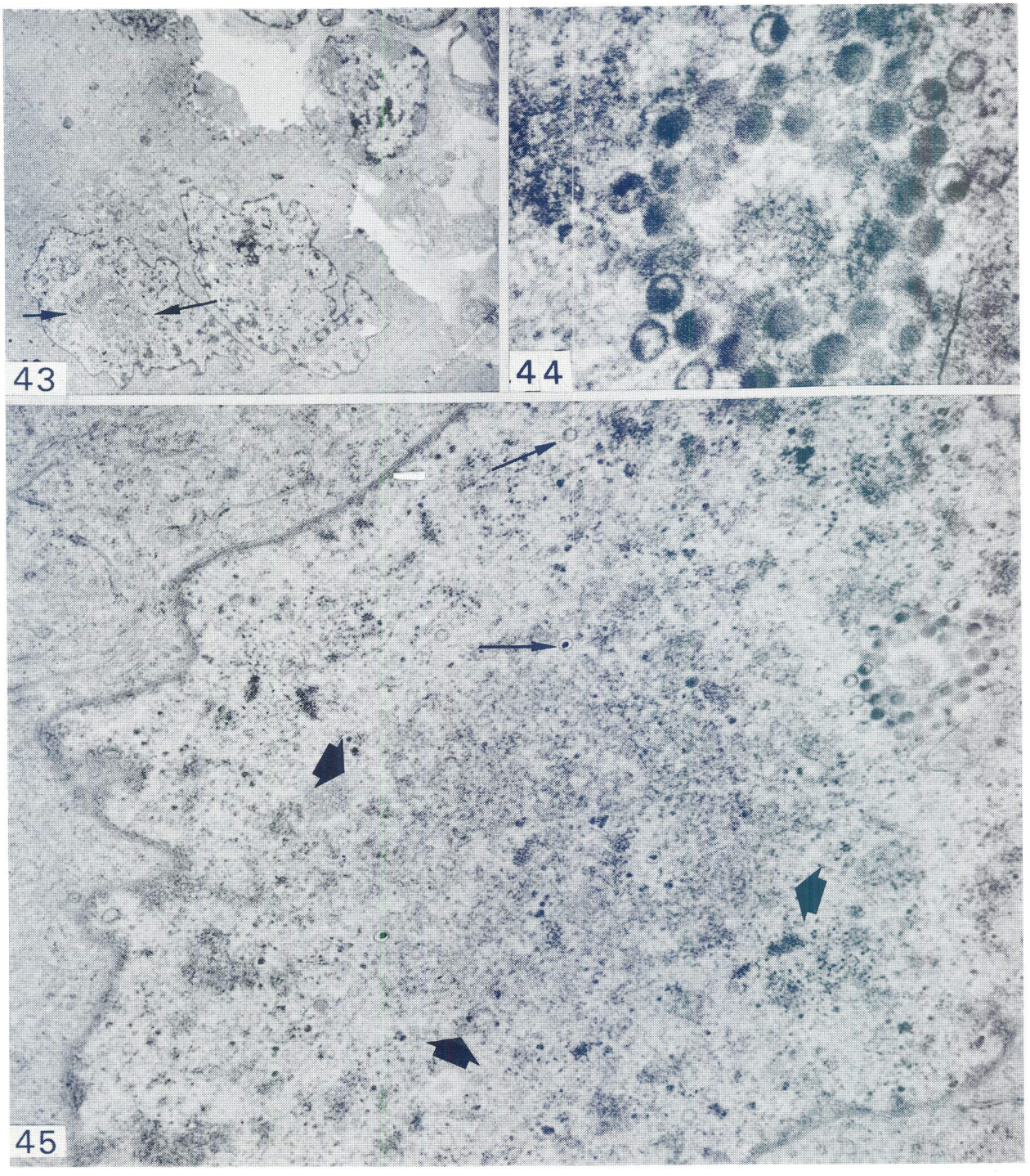

FIG. 8 43. Low magnification of a giant cell. The nucleus on the left contains an inclusion body (arrows) × 4 250 44. A cluster of virus particles consistent with the herpesvirus group within the nucleus of a giant cell. The diameter of the particles is 100 nm. × 80 000 45. Scattered single virus particles (small arrows) can be seen in and around the finely granular inclusion body (large arrows) plus a cluster of virus particles present on the right. × 28 800

this virus in the elephant result from its effect on two types of cells, namely lymphocytes and the epithelial cells lining the alveoli. The changes in the latter are apparently due to the direct effect of the virus on the cell, but the lymphocytic response is probably of a secondary nature. These changes can best be discussed separately.

At first the alveolar lining cells hypertrophied and became more epithelial in appearance. Secondly, proliferation with various patterns of growth and formation of syncytial giant cells with Cowdry Type A intranuclear inclusions occurred. Hyperplasia, as induced by pox viruses, and the formation of syncytial giant cells, such as those seen in the lesions of herpes viruses, are two wellknown effects of viruses on epithelium.

In many viral diseases concentrations of lymphocytes are found in abnormal locations. The perivascular accumulation of lymphocytes in the brain in many viral encephalitides and in the meninges of animals with lymphocytic choriomeningitis and other diseases are examples of this phenomenon. The lymphoid nodules with typical Cowdry Type A inclusions in the lung of the elephant are considered to be pathognomonic of this herpes virus disease. The organization of the lymphoid cells into nodules in the elephant lung is remarkable but by no means unique. In lymphocytic choriomeningitis the response appears to be due to the stimulus of the virus present in the lymphoid accumulations. In the elephants it appears that the virus is, initially at least, in the alveolar lining cells. Thus a situation arises in which epithelial cells containing intranuclear inclusions due to virus are surrounded by masses of lymphocytes. An analogous situation may be seen in cytomegalo virus infection, also a type of herpes virus, where the virus and inclusions are present in epithelial cells amidst lymphoid hyperplasia. Strano (AFIP, Washington, D.C., personal communication, 1971) has seen lymphoid follicles in the accumulations of lymphocytes in mice having cytomegalo virus infection.

Apparently the herpes virus in the elephant, unless complicated by secondary infections, usually exists as a subclinical or latent infection and its incidence is probably close to 100% in certain herds. Hence one may possibly expect overt disease only in young elephant calves. Hunt & Meléndez (1969) noted that such phenomena are general characteristics of the host-parasite relationship of herpes virus infections in the reservoir host(s). They also emphasized that the herpes viruses in any but their usual host(s) are almost invariably fatal. Some of the examples given were *Herpes virus suis,* latent in swine but fatal in cattle, *Herpes virus hominis,* latent in man but fatal in owl monkeys and *Herpes virus B,* latent in rhesus monkeys and fatal in man. In the host in which it is either fatal or the most pathogenic, the virus may be pantropic. Thus it is expected that this herpes virus of the elephant may be highly pathogenic in one or more of the domestic animals or man. This remains to be determined.

Various authors have called attention to the value of viral inclusions in the diagnosis of diseases. Habermann, Williams & Fite (1960) compiled a table which included over 50 such viral diseases, some of which affected several animal species. Subsequently, numerous other viral diseases associated with inclusions have been reported. It is now evident that at least one herpes virus of the elephant occurs which can be added to this list. Goodheart (1970) recently also reviewed the role and/or possible roles that certain viruses, notably herpes viruses, play in carcinogenesis. Any new herpes virus should therefore be scrutinized with all the pathogenic potentialities in mind.

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