HISTOPATHOLOGY OF INFECTIOUS LARYNGOTRACHEITIS IN CHICKENS

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(Received for publication, July 28, 1931)

Since the first report of the occurrence of laryngotracheitis by May and Tittsler (1), this disease has been studied and described in various parts of the United States by Eriksen (2), Hinshaw (3), Beach (4), Graham (5), and Kernohan (6). They have dealt with the clinical symptoms, the gross pathological findings, and the infectious agent, which has been definitely proven by Beach (7) to be a filtrable virus. No histological study has been published. It is the purpose of this paper to report such studies, which were started in January, 1930, and continued in close collaboration with the etiological and experimental work of Beach.

Material

The material consisted of thirty cases of laryngotracheitis, of which fourteen were experimental and sixteen spontaneous field cases. Some of the fowls were killed with chloroform between 6 hours and 7 days after inoculation while others died in various stages of the disease. The field material and the virus with which fowls were inoculated experimentally were obtained from both New Jersey and California outbreaks.

Animals were inoculated intratracheally, intranasally, into the eye, or into the cleft palate. A few were allowed to contract the disease through contact with infected chickens. The disease picture was the same after all types of inoculation except into the nasal fossa, the eye, and the cleft palate. Infection by these routes led to the development of a more or less marked discharge from the nose, eyes, or cleft palate in addition to the typical laryngotracheitis.

Gross Pathology

Larynx and Trachea.—At autopsy the most striking lesions were regularly found in the upper larynx, the trachea, and less frequently the lower larynx. 6 to 42 hours after inoculation only a small amount of mucus was visible. Between 42 and 74 hours after inoculation there might be small hemorrhages in the mucous membrane or a small amount of mucus mixed with clotted blood and restricted entirely to the upper larynx; while 3 or more days after inoculation the mucus might be replaced by caseous, yellowish masses which collected in the larynx or trachea to such an extent that they entirely plugged the opening. The mucous membrane of the lower trachea was normal except for a slight swelling, reddening, and increase of mucus which covered it with a thin film. In most of our cases this process extended the entire length of the trachea and often into the main bronchi. The degree and the kind of lesions varied greatly. Frequently the mucous membrane was covered with a comparatively thin film of clotted blood, with mucofibrinous and caseous masses, or with a mixture of blood and exudate; while in other cases clotted blood or exudate was collected in a mass so large that it completely occluded the lumen of the lower portion of the trachea and of the main bronchi. Sometimes the amount of clotted blood exceeded that of mucus. Cross-sections through the larynx, trachea, and bronchi showed an enormous thickening of the mucous membrane.

Except for a more or less marked congestion and edema the lungs appeared entirely normal in most of our cases. In a very few a yellowish exudate between the chest wall in either or both lungs and foci of consolidation and necrosis of a yellowish color were present.

Nasal Passages and Sinuses.—A number of chickens which contracted the disease naturally or were inoculated in the nasal fossae, cleft palate, or eyes showed, in addition to the described lesions, an involvement of the upper respiratory tract. The turbinate bones and the paranasal sinuses were sometimes filled with seromucoid, water-clear or yellowish caseous material, which by application of a slight pressure could be forced out of the nostrils and cleft palate. This involvement of the nasal passages and the sinuses was often indistinguishable from that found in the common fowl diseases, A-avitaminosis, pox, roup, and diphtheria. The same inflammatory process might be present in the eyelids.

Alimentary Tract.—In a large percentage of cases, even in very early ones, the mouth cavity contained small thin patches or films, either round or oval, which could be removed without leaving defects in the underlying mucous membrane. These lesions, although easily distinguished from those found in diphtheria, are also found in common roup and A-avitaminosis and are therefore not characteristic of laryngotracheitis.

No significant gross lesions have been found in any part of the intestinal tract. Some cases showed a slight inflammation of the small intestine.

Spleen.—In a few cases the spleen appeared slightly enlarged.

Other Organs.—No gross lesions were encountered in other organs. In a number

of birds in which occlusion of the trachea or bronchi caused death by asphyxiation, small hemorrhages were found in the epicardium.

Histology

Larynx and Trachea.—24 to 72 hours after inoculation the predominant picture is a more or less marked edema and cellular infiltration of the tunica propria and the submucosa (Fig. 1). In some of our cases the edema of the mucous membrane causes an entire disarrangement of its normal structure (Fig. 2). The inflammatory process may even spread into the adventitia. One of the most noticeable lesions is the presence of small hemorrhages around smaller vessels of the propria mucosa and submucosa or such an extravasation of the red blood corpuscles that the mucosa and submucosa are completely separated from each other. A few red blood corpuscles also occur throughout the upper part of the edematous mucous membrane and even appear on its surface. In view of the negative histological findings in the walls of the blood vessels in these early stages, one is led to believe that the escape of cells is due to rupture of smaller vessels of the submucosa caused by violent coughing and respiratory efforts or possibly by diapedesis.

In later stages of the disease (3 to 7 days after inoculation) the lesions in the larynx and trachea are characterized by a dense cellular infiltration in both tunica propria and submucosa (Fig. 3). From the comparative study of a number of cases it becomes evident that the larynx as a rule shows more infiltration than the trachea and bronchi. While in some of the cases the normal structure of the mucous membrane and the mucous glands can still be recognized, in the majority there is such a dense infiltration that the epithelial elements become more or less dislocated and destroyed.

The greater number of the infiltrating cells are small lymphocytes, with histiocytes, and a few plasma cells. The proportion of the different kinds varies; while in some cases the lymphocytes are most numerous, the histiocytes predominate in others. Eosinophilic leucocytes, present normally in the trachea of chickens, may be increased in number. Mitotic figures indicate a slight increase of the connective tissue elements. In sections impregnated by Bielschowsky-Maresch's silver method, connective tissue fibers are found to be increased. The fibers are swollen and sometimes broken as a result of the enormous edema. The same is true of the elastic tissue fibers. The blood vessels show no distinct and regular lesions except for a more or less marked swelling of the endothelial cells and a change in the chromatin structure of their nuclei. In some cases retrogressive changes are found in the connective tissue cells of the submucosa, which may contain small fat droplets in their protoplasm and nuclei. This degenerative process does not usually reach a high degree during the first 5 days after infection. Older cases, however, frequently show actual necrotic foci in the submucosa and tunica propria with a general hyperchromasia, pycnosis, and karyorrhexis of nuclei, and fatty degeneration of cells including those of the various infiltrating elements. In these late cases and sometimes also in earlier ones, a marked proliferation of endothelial cells

with necrosis of the walls of the blood vessels is present. These lesions, when they occur, may provide an entrance for the virus into the blood stream and its conveyance to the liver and spleen in which, according to Beach (7), it is found frequently.

While in some very early cases the epithelium of the mucous membrane and of the mucous glands retains its normal structure and the individual cells are almost normal in appearance, more or less marked changes can usually be seen as early as the 6th hour after inoculation. The first change is a slight thickening of the surface epithelium caused by the general edema of the wall of the trachea, enlargement of individual cells, and occasionally multiplication of cells (mitotic figures). Some of the cells may appear practically normal, but the majority of those lining the upper surface lose their cilia and are thrown off, and show retrogressive changes, oftentimes the so called "balloon degeneration." Reticular degeneration of epithelial cells in the surface layers is a rare finding. In most of the cases showing a marked edema of the mucous membrane there is a disarrangement of the various parts of the tracheal wall, and in those with a pronounced infiltration the epithelium, including the mucous glands, is nearly destroyed (Fig. 3).

Formations simulating giant cells occur in the edematous fluid between the surface epithelium and the submucosa (Fig. 2). By the use of Mayer's mucicarmine stain they can be demonstrated to be mucous glands separated from the surface epithelium and scattered irregularly in the edematous mucous membrane. They are always found compressed by the surrounding edematous fluid and show degenerative changes of the peculiar type described above. Later the nuclei disintegrate, the degenerative products collect in the center, and there is a hyperchromasia and hypereosinophilia. Finally the whole formation may be transformed into a necrotic area in which mucin can still be demonstrated with specific stains. Fat may be present in large amounts.

The individual variation in the findings is great, depending upon the length and severity of the process. Secondarily invading bacteria may lead to a partial or diffuse necrosis of the tracheal wall and complicate the picture. Cocci and more frequently diphtheroids, probably similar to those described by Graham and his coworkers (8), are present in such cases. In general, however, such bacteria seem to play a minor rôle. Rickettsias have not been found.

Tracheal Exudate.—The composition of the exudate in the trachea differs widely in individual cases. In the early stages there is only a slight proliferation of the upper layers of epithelial cells which are thrown off either singly or in layers and collect on the surface of the mucous membranes; while in other cases there is a mixture of epithelial cells, mucus, round cells, eosinophilic leucocytes, and red blood corpuscles. Sometimes the cellular elements predominate, sometimes the mucus. There are cases in which the mucous membrane is covered with a thin layer of red blood corpuscles. In other cases what appears to be exudate is really edematous mucous membrane which has become separated from the underlying tissue and thrown off in large masses. It is a striking fact that fibrin could seldom be demon-

strated in the exudate. In a number of cases the exudate consists of degenerated cells and sometimes real necrosis is seen. In instances with marked infiltration of the mucous membrane exudate may be entirely lacking.

Lungs and Bronchi.—The changes in these organs are usually restricted to the bronchi and the tubules. The bronchitis, which is almost always present, varies in degree and is often associated with a peri- and parabronchitis. The lesions resemble those in the larynx and trachea but very seldom are as marked. The bronchial exudate is largely made up of the same elements and shows the same variations as that in the trachea. Sometimes bronchiectasis is present. In addition to these lesions, in almost all cases there occurs marked congestion indicated by an extreme filling of the capillary network of the lungs and a more or less pronounced edema (Fig. 4). Scattered around the hemorrhages in the bronchial wall and in the congested areas are groups of hemin crystals either in the tissue or included in various cellular elements.

These lesions in the lungs and bronchi seem to be more extensive in the spontaneous disease than when it is artificially produced. In a few cases a true pneumonia is present in the tissue surrounding the affected bronchi. In addition more profuse hemorrhages and larger foci of necrosis are found in the lung parenchyma, often walled off by fibroblasts. In many cases numerous foreign body giant cells are found among the fibroblasts (Fig. 5).

Nasal Cavities and Communicating Sinuses.—The lesions in the nasal passages and the communicating sinuses vary considerably. In general, they are analogous to those previously described in the trachea (Fig. 6). Lesions due to bacterial infection are just as rare as in the trachea and bronchi.

Eyelids and Eyes.—In natural and experimental cases with involvement of the eyes, the eyelids show the same inflammatory process as the trachea. No lesions are present in the other parts of the eye.

Alimentary Tract.—The small yellowish membranes frequently seen in various parts of the cavity of the mouth are necrotic areas, sometimes very superficial, sometimes destroying the entire epithelial layer of the mucous membrane. Numerous clumps of bacteria are found which are probably the cause of the lesions. They occur in a number of other diseases, and hence cannot be considered characteristic of the infection under discussion.

The esophagus, proventriculus, and intestinal tract are free from lesions except, in a few cases, for a slight enteritis which does not seem to be specific.

Spleen.—The spleen when enlarged shows a hyperplasia without any other particular or characteristic lesions.

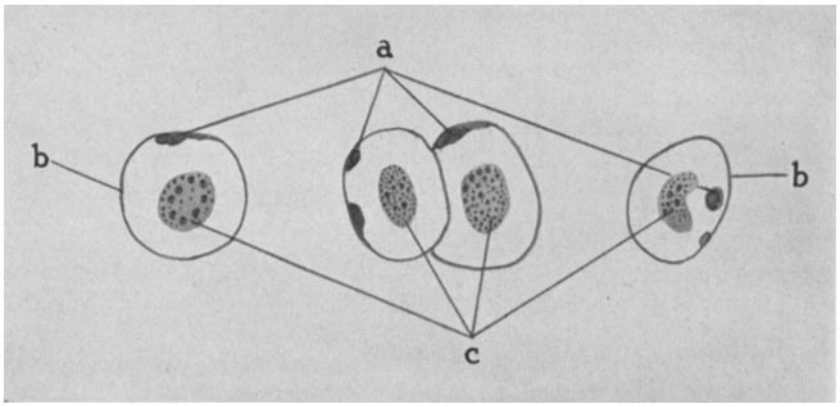
Liver, Kidney, and Heart.—In practically all of our cases we found in the liver more or less marked infiltrations with round cells and eosinophilic leucocytes, and sometimes proliferations around blood vessels and gall ducts. The kidneys exhibit in a few cases small hemorrhages and infiltration with round cells without significant changes in the glomeruli, convoluted and collecting tubules. In a few cases the same type of infiltration is encountered in the interstitial tissue of the heart

muscle. It is impossible to establish any relationship between these lesions and the activity of the virus of infectious laryngotracheitis, because lesions of the same kind occur in the kidneys and heart of "normal" chickens.

The remaining organs are free from lesions.

Intranuclear Inclusions in Epithelial Cells of the Respiratory Tract

In early stages of infectious laryngotracheitis characteristic intranuclear inclusions and nuclear changes could be demonstrated in the epithelial cells lining the mucous membrane as well as in those of the mucous glands of the trachea, and less frequently in those of the nasal



TEXT-FIG. 1. A drawing of four nuclei of tracheal epithelial cells. (*a*) Nucleoli; (*b*) nuclear membrane; (*c*) intranuclear inclusions showing argentophilic granules of various sizes and arrangement. Klarfeld's tannic acid and silver impregnation.

passages (9). They appeared to be characteristic of the disease. In some instances smaller acidophilic bodies were shown in otherwise unchanged nuclei, but usually the nuclei showing the typical changes exhibited a more or less marked enlargement and hyperchromasia of the nuclear membrane, which caused it to appear sharply outlined. These inclusions, homogeneous in appearance, were usually situated centrally, and occupied a large part of the nucleus (Fig. 7, Text-fig. 1). They were usually round or oval, sometimes long and sausage-shaped, and occasionally had the form of a diplococcus. They were sharply outlined and took a pink stain with Giemsa's stain, while they stained

red with methylene blue-phloxin, hematoxylin-safranin, Goodpasture's fuchsin-methylene blue, and Gerlach's stain for Negri bodies. The nucleoli, of which these cells generally possessed one to three, were located peripherally, often attached to the nuclear membrane, and stained very intensely with basophilic stains. Another striking and characteristic feature was that the space between the nuclear membrane and the inclusion remained entirely unstained (Fig. 7, Text-fig. 1). They appeared as early as 12 hours after inoculation and more often occurred in all of a group of epithelial cells than in a single one. The protoplasm of the cells showing them stained very slightly, was more or less vacuolated, and the cell boundaries were no longer distinguishable (Fig. 7). The appearance and size of the intranuclear bodies as well as the changes in the nuclei bore a close resemblance to those described in herpes, varicella, virus III of rabbits, and submaxillary gland disease of guinea pigs (10). The precise character of the intranuclear acidophilic substance has not been ascertained. It did not stain with the fat stains or give the iron reaction, but it showed a slightly positive Feulgen reaction for thymonucleic acid (11). In using various silver stains numerous small argentophilic granules could be revealed inside the inclusions (Text-fig. 1). It is a striking fact that in many instances the affected nuclei underwent dissolution. We have not observed similar changes of epithelial cells in other diseases involving the same structures, such as A-avitaminosis, coryza contagiosa, and fowlpox (12, 13). Since the same type of intranuclear inclusions and nuclear changes are present in other virus diseases, we feel justified in considering them a result of virus activity in the epithelial cells of the trachea and larynx.

DISCUSSION

In spontaneous and experimentally produced cases of laryngotracheitis the lesions are most pronounced in the larynx and upper part of the trachea. Because the histological findings correlate with the clinical symptoms and the gross anatomical picture, we believe that the name "infectious laryngotracheitis," as used by Graham, is most truly descriptive and should therefore be adopted. Our findings indicate furthermore that the involvement of the nasal passages, communicating sinuses, and eyelids depends upon the mode of infection and the

course of the disease. The virus, which has thus far in Beach's investigations proven pathogenic for chickens only, seems to possess a distinct organ selectivity, being limited in its activity to the mucous membranes of the respiratory tract. Though it occasionally enters the blood stream it does not lead to characteristic lesions in other tissues.

As in many other virus diseases, the agent producing infectious laryngotracheitis causes an inflammatory process with edema or cellular infiltration, followed by retrogressive changes and a certain amount of proliferation. The course of the disease is so rapid that it is extremely difficult to determine precisely the location and the character of the primary injury and to follow the development of the lesions and the order of their occurrence. This difficulty is rendered still greater by the complexity of the mucous membrane of the respiratory tract. By a careful study of cases killed 6, 12, and 24 hours after intratracheal inoculation with a small dose of virus, it has been found that lesions of the surface epithelium may be present without notable changes in the submucosa. Films made with the mucus covering the mucous membrane of the trachea in these cases show a great many desquamated epithelial cells with various kinds of degeneration. Red blood corpuscles are also found in films from the mucous membrane of these cases. The conclusion seems justified that the virus attacks the epithelial cells first, penetrating afterwards to the submucosa. The inflammatory process occurring in this layer is of such severity as to constitute the predominating phenomenon. The destruction taking place later in the disease can be attributed not only to the virus but to mechanical pressure from edema, accumulations of infiltrating cells, or hemorrhages; and occasionally to the presence of secondary invaders.

The finding of intranuclear inclusions resembling those in herpes, varicella, virus III of rabbits, and submaxillary gland disease of guinea pigs, adds another disease to those in which such forms are associated with viruses. In laryngotracheitis they are present only in the earlier stages of the disease, which may be considered evidence in favor of their relationship to the virus.

SUMMARY AND CONCLUSIONS

1. The characteristic lesions of infectious laryngotracheitis are ordinarily restricted to the respiratory tract and are most pronounced in

the larynx and trachea. Sometimes the eyelids are affected. A certain percentage of the cases are associated with bronchitis and peribronchitis, pneumonic areas and hemorrhages in the lung, while the involvement of the nasal passages, communicating sinuses, and eyes seems to be dependent upon the mode of infection and the course of the disease.

2. The virus affects the epithelial cells primarily, but soon inflammation develops in the submucosa and underlying parts. Edema is often extremely pronounced in the submucosa. The destruction taking place at later stages is due to edema, cellular infiltration, and hemorrhages, and in some instances to secondarily invading bacteria.

3. Characteristic intranuclear inclusions in the epithelial cells of the trachea are present in many cases. They bear a close resemblance to the inclusions occurring in herpes, varicella, virus III of rabbits, and submaxillary gland disease of guinea pigs.

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EXPLANATION OF PLATES

PLATE 58

FIG. 1. Cross-section through trachea of a chicken killed 2½ days after intratracheal inoculation. Infiltration of the submucosa and mucosa, thickening of epithelial layers, and proliferation and degeneration of cells at the surface. Hematoxylin-eosin. ×136.

FIG. 2. Cross-section through trachea of a chicken killed 3½ days after intratracheal inoculation. Destruction of the surface epithelium, marked edema and slight infiltration of the entire mucous membrane, separation and destruction of glands. Hematoxylin-eosin. × 136.

FIG. 3. Cross-section through larynx of a spontaneous case. Round cell infiltration and enormous thickening of the mucous membrane. Destruction of the surface epithelium and that of the glands. Hematoxylin-eosin. × 136.

PLATE 59

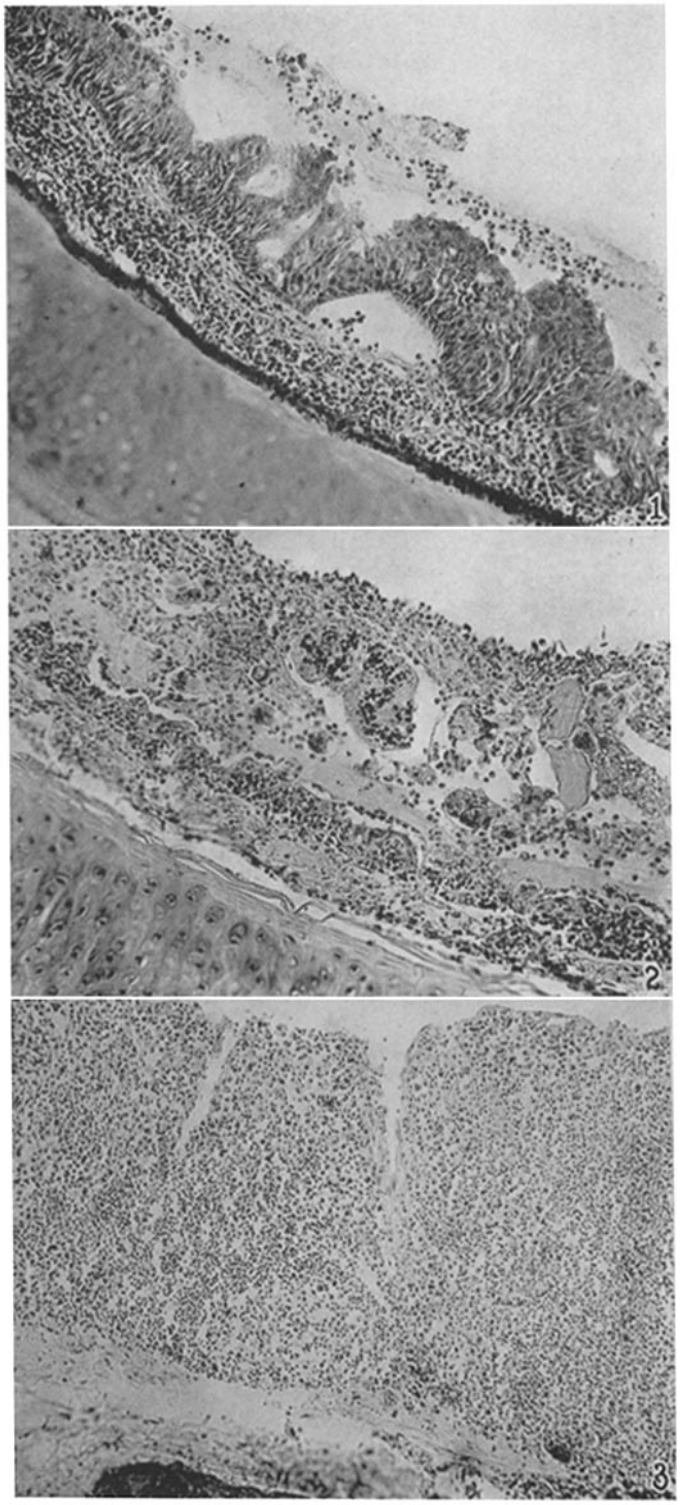
FIG. 4. Lung from chicken dead 6 days after intratracheal inoculation. Bronchitis and edema. Hematoxylin-eosin. × 180.

FIG. 5. Necrotic hemorrhagic focus in the lung walled off by the formation of fibroblasts and giant cells. Chicken dead of spontaneous disease. Hemotoxylin-eosin. × 184.

PLATE 60

FIG. 6. Turbinate. 4 days after inoculation into cleft palate. Inflammatory process of the nasal mucosa. Hematoxylin-eosin. × 184.

FIG. 7. Group of tracheal epithelial cells from a spontaneous case. A large proportion of the nuclei contain characteristic inclusions. In some of such nuclei the nucleoli are attached to the nuclear membrane. Giemsa stain. × 1000.



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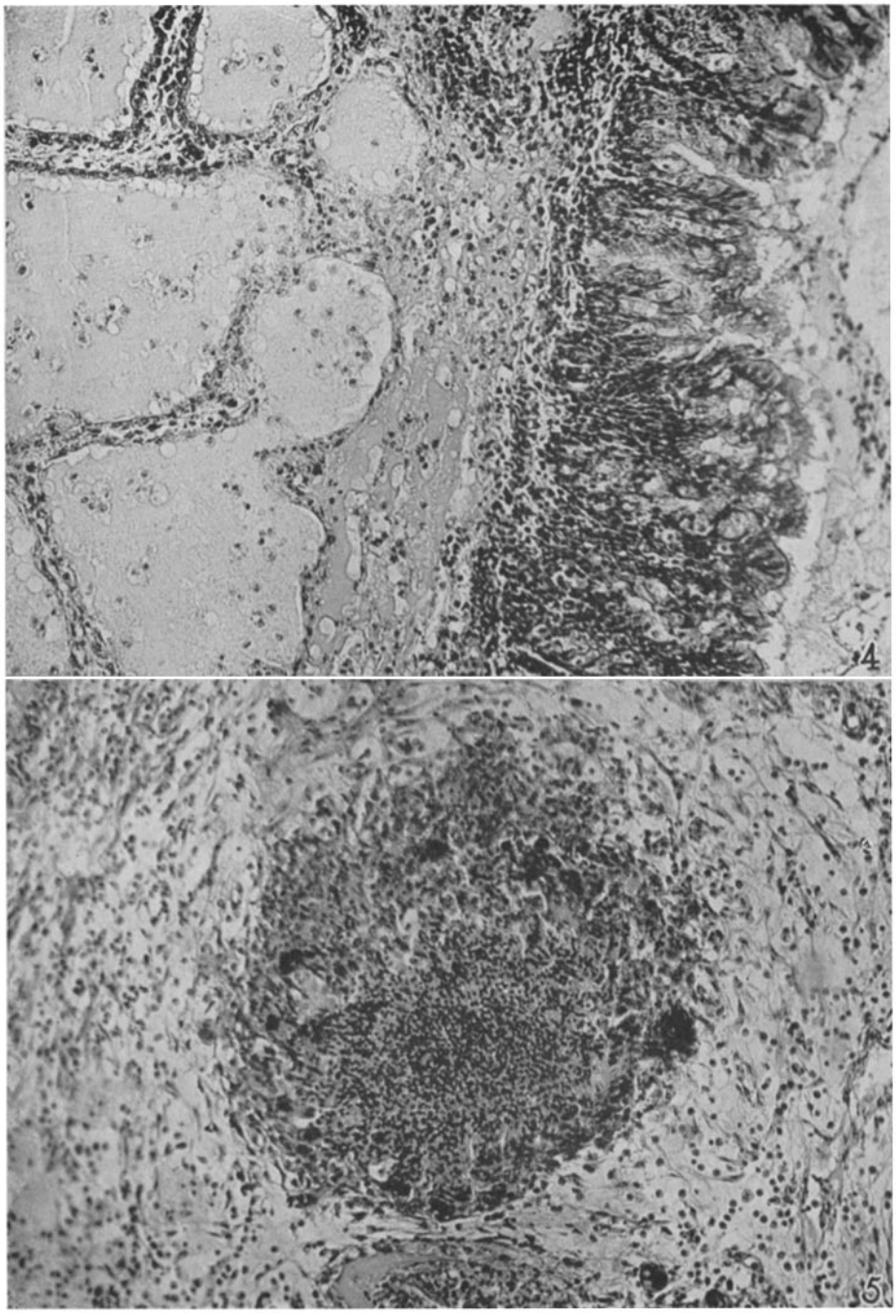

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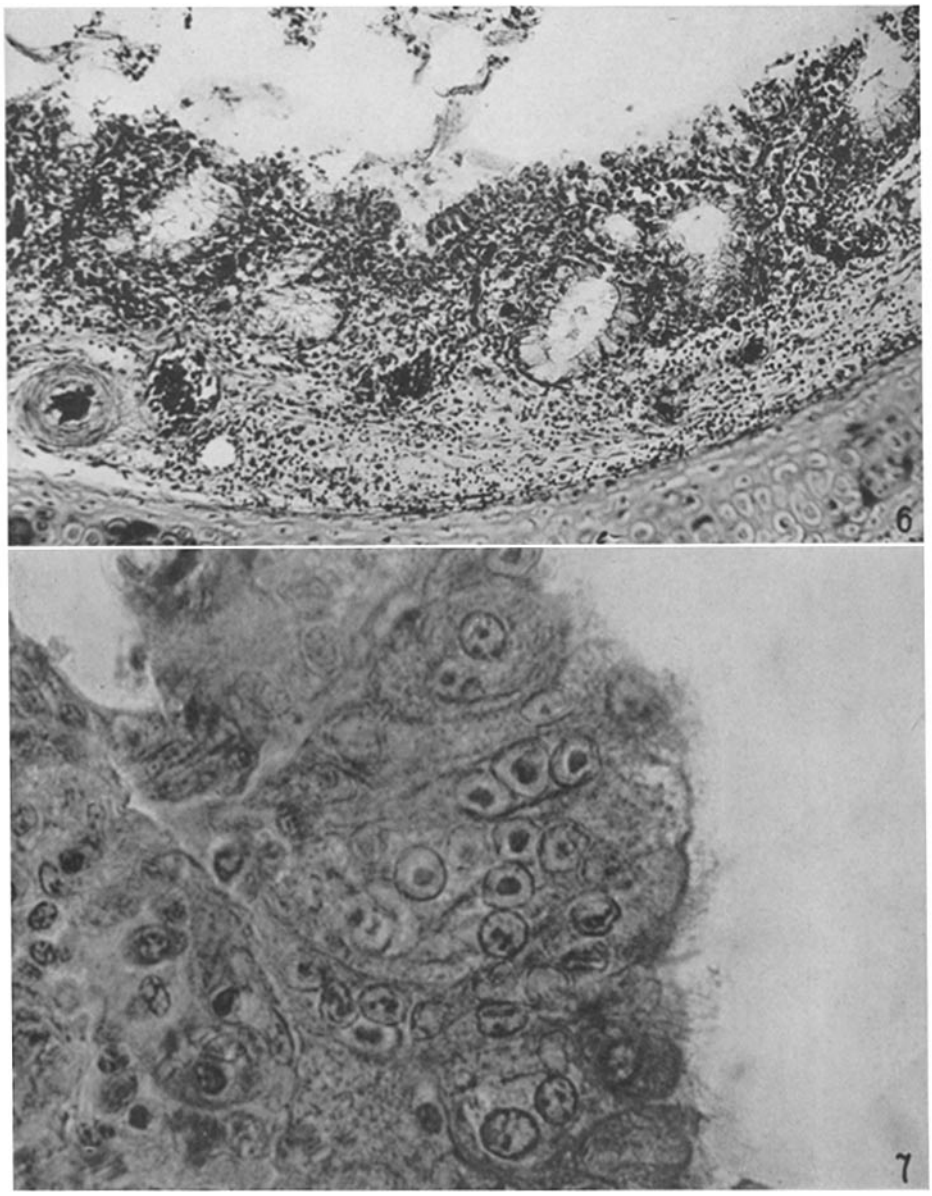

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