EXPERIMENTAL STUDIES OF THE NASOPHARYNGEAL SECRETIONS FROM INFLUENZA PATIENTS.

V. Bacterium pneumosintes and Concurrent Infections.

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Plates 1 to 3.

(Received for publication, March 1, 1921.)

In the preceding article[1] of this series, we described the anaerobic, filter-passing organism which has been cultivated by special methods from the nasopharyngeal secretions of patients in the early hours of uncomplicated epidemic influenza, and from the lungs of rabbits and guinea pigs experimentally inoculated with these secretions. Earlier experiments had demonstrated the presence, in the nasopharyngeal secretions, of an active agent of peculiar character, distinguished from ordinary bacteria by its effects on the lungs and blood of experimental animals, by filter passage, and by resistance to glycerolation for a period of months.[2,3] Parallel experiments with the anaerobic organism disclosed a similarity of biologic properties and pathogenic effects sufficient to establish its identity with the active agent. We have, therefore, stated[1] that the pathogenic activity of the nasopharyngeal washings from early cases of uncomplicated epidemic influenza, as tested in our experimental animals, was due to the presence of the anaerobic, filter-passing organism which we have recovered from these secretions.

A peculiar and significant property of the active agent, in view of its origin, was its effect in reducing the resistance of the lung tissues of inoculated animals to accidental or experimental infection with bacteria of ordinary species—those bacteria for example which were so

[1] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 713.
[2] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 125.
[3] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 361.

frequently found in concurrent or secondary pneumonias associated with epidemic influenza in man.[4]

If the active agent in influenzal secretions has been identified in the filter-passing organism, it follows that this organism should have the same property of reducing pulmonary resistance in experimental animals. It is the purpose of the present paper to describe our observations and experiments bearing on this point.

Accidental Concurrent Infections with Ordinary Bacteria.

Under the heading of accidental infections may be grouped those scattered experiments in which the intratracheal inoculation of a washed mass culture of the filter-passing organism was followed by a pulmonary infection with ordinary bacteria. We have already described such accidents after inoculation of the active agent from influenzal washings.[4] These experiences were less frequent in the later experiments with the filter passer, due to the avoidance of oral contamination after the catheter method first employed was discarded in favor of needle puncture of the exposed trachea.[5]

Nevertheless, ordinary bacteria were encountered in the lungs of six rabbits in five series of transmission experiments with cultures originally derived from a strain of the filter-passing organism obtained from a case in the first, or 1918–19, epidemic. Three other transmission series remained uncomplicated throughout. In these experiments the first passage was initiated with a culture of the filter passer. Subsequent passages were effected with suspensions of lung tissue from the preceding rabbits. Thus the organism was carried through as many as five rabbit passages, and subsequently recovered.

Bacillus welchii was recovered from two successive rabbit passages, the fourth and fifth, of one transmission series. *Bacillus coli*, *Bacillus lepisepticus*, *Staphylococcus aureus*, and a large Gram-positive bacillus were each recovered once (Table I). The occurrence of such an accidental infection caused the termination of the series.

The presence of a concurrent bacterial infection in these rabbits was usually indicated by aggravated symptoms and by prostration

[4] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 373.

[5] All operations were performed under light ether anesthesia.

and death within 48 hours if the animal was not killed earlier. An intense injection or a purulent inflammation of the conjunctivæ developed. Loss of weight was marked. The blood picture showed a greater depression of the leucocytes, involving both the polymorphonuclear and the mononuclear cells than that occurring in the animals which were injected with *Bacterium pneumosintes*. In the rabbits in which *Bacillus coli* and the Gram-positive bacillus were encountered, the initial depression was followed by a polymorphonuclear leucocytosis.

TABLE I.

Occurrence of Ordinary Bacteria in the Course of Transmission Experiments with B. pneumosintes.

Generation of *B. pneumosintes* beginning the series.	No. of rabbit passages showing typical effects.	Rabbit passage showing secondary infection.	Kind of bacteria.	Pathological effect in rabbit.
Second.	5	Second (only in one of two rabbits).	*Staphylococcus aureus.*	Diffuse polymorphonuclear consolidation with abscesses.
Fourth.	2	Second.	*B. coli.*	Abscess of lungs.
"	2	" (only in one of two rabbits).	Large Gram-positive aerobic bacillus.	" " "
Eighth.	5	Fourth. Fifth.	*B. welchii.* " "	Bronchopneumonia. "
Ninth.	4	Fourth.	" *lepisepticus.*	Purulent bronchitis; patchy pneumonia.

At autopsy the familiar pulmonary lesions—hemorrhagic edema and emphysema without consolidation or pleuritis—were complicated or masked by other lesions attributable to the ordinary bacteria involved. A diffuse polymorphonuclear exudation was accompanied by patchy or lobar consolidation, localized small abscesses, and necrosis of the vascular endothelium with thrombus formation. A purulent bronchitis resulted from the *lepisepticus* infection. From these lesions the invading organisms were cultivated and identified.

Thus the course of accidental bacterial infection in rabbits inoculated with the filter passer closely paralleled the findings in similar infections accompanying the active agent of the earlier experiments. A more exact basis of comparison is afforded by parallel experiments with the active agent and the filter-passing organism in which concurrent or secondary bacterial infections were experimentally induced.

Experimental Concurrent Infections.

With the exception of *Staphylococcus aureus*, the accidental invaders in our experiments do not belong to the group of organisms commonly found in concurrent or secondary pneumonias so frequently associated with epidemic influenza in man. For the production of experimental concurrent infections we therefore chose two organisms as examples of the frequent inhabitants of the nasopharynx which have been recovered from many postinfluenzal pneumonias, *Bacillus pfeifferi* and a Type IV pneumococcus.

We have already described[4] the results of intratracheal or intravenous injection of these organisms alone in the small doses employed in the following experiments. Their effects were transient and differed essentially from the equally transient effects of the influenzal active agent. It was the combined action of bacteria and active agent in the same rabbit which produced the fatal pneumonias in the experimental animals and led us to point out the significant similarity of these pneumonias to those associated with epidemic influenza in man. With these earlier experiments as a basis of comparison we were now ready to study the effects of the ordinary bacteria in combination with *Bacterium pneumosintes*.

Rabbits were first inoculated intratracheally with the cultivable bodies and then received suitable doses of the chosen bacteria either intratracheally or by an ear vein.

The following protocols illustrate the results of the injection of cultivable bodies and ordinary bacteria by the intratracheal route.

Protocol 1.[6] *B. pneumosintes and Pneumococci.*—Nov. 17, 1920. A rabbit whose normal temperature was 38.9°C., total leucocytes 11,000, of which 4,620 were mononuclears, was inoculated intratracheally with the washed sediment of a

[6] Only typical protocols of a number of similar experiments are presented.

fourth generation mass culture[7] of cultivable bodies originally derived from the 1918–19 epidemic. Nov. 18. Temperature 39.3°C., total leucocytes 8,600, of which 1,892 were mononuclears. Inoculated intratracheally with one loopful of a 48 hour growth of Type IV pneumococcus on a standard agar slant. Nov. 20. Temperature 40°C., leucocytes 6,000, of which 2,400 were mononuclears (Text-fig. 1). Killed. The lungs showed lobar consolidation (red hepatization) of the right upper and lower lobes and a small patch of consolidation in the left upper lobe. The lesion in the left lower lobe consisted of edema, emphysema, and a number of small hemorrhages. Film preparations of the consolidated area showed 80 per cent polymorphonuclear cells and a few pneumococci. Aerobic cultures yielded Pneumococcus Type IV.

Control rabbits were separately injected with the same doses of *B. pneumosintes* and the pneumococcus. In the first instance the injection was followed in 48 hours by a fall in the total leucocyte count, mainly due to a drop in the mononuclears. The rabbit was killed. At autopsy the lungs showed a typical hemorrhagic edema and emphysema without consolidation. Aerobic cultures remained sterile. Anaerobic cultures yielded *B. pneumosintes* in pure culture.

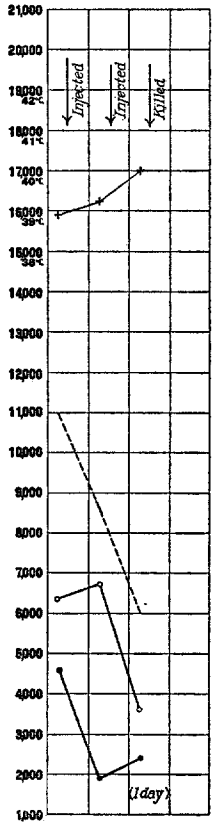
The second control rabbit, injected intratracheally with one loopful of pneumococcus culture, was killed after a similar interval. The lungs showed no lesion. Aerobic cultures were free from growth.

Protocol 2. B. pneumosintes and B. pfeifferi.—Dec. 11, 1920. A rabbit whose normal temperature was 39.2°C. and total leucocytes 7,000, of which 3,850 were mononuclears, was inoculated with the washed sediment of a seventh generation mass culture of cultivable bodies originally derived from the first, or 1918–19, epidemic. Dec. 12. Temperature 39.5°C. Total leucocytes 4,600, of which 794 were mononuclears. Inoculated intratracheally with the 24 hour growth on a blood agar slant of *B. pfeifferi.* Dec. 13. Temperature 39.5°C. Total leucocytes 10,000, of which 2,500 were mononuclears (Text-fig. 2). Killed. The lungs showed pneumonic consolidation of the right and left upper lobes, and edema, emphysema, and patchy hemorrhages in the other lobes. The diffuse polymorphonuclear exudation in the consolidated areas is shown in Fig. 1. Aerobic cultures yielded *B. pfeifferi.*

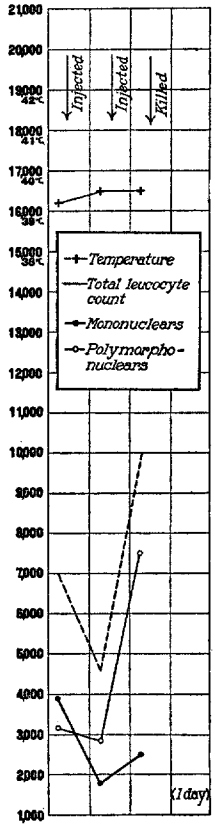
A control rabbit, inoculated intratracheally with the same dose of *B. pneumosintes* showed the clinical and pathological effects regarded as typical and already described at length[1] (Fig. 2).

A second control rabbit was injected intratracheally with the washed sediment from an uninoculated control of the mass culture medium, and on the following day with the same dose of *B. pfeifferi* that was given to the experimental animal. This control rabbit was killed 24 hours later. The lungs showed no lesions. Aerobic cultures yielded no growth.

[7] The dose and preparation of the growth in mass culture for inoculation are described in another paper.[1]



TEXT FIG. 1.



TEXT FIG. 2.

TEXT-FIG. 1. Effect on blood count and temperature (Protocol 1). The first intratracheal injection consisted of the cultivable bodies and was followed by a rise in temperature and depression of the total leucocytic count, caused by a deficiency of mononuclears. The second intratracheal injection, of pneumococci, caused no essential change in temperature or blood count.

TEXT-FIG. 2. Effect on blood count and temperature (Protocol 2). The first intratracheal injection consisted of the cultivable bodies and was followed by a rise in temperature and depression of the total leucocytic count, caused by a deficiency of mononuclears. The second intratracheal injection, of *B. pfeifferi*, caused a polymorphonucleosis.

The injection of cultures of the cultivable bodies, followed by subinfective doses of pneumococci or Pfeiffer's bacilli, produced a lobar or bronchopneumonic consolidation of the lungs with polynuclear exudation, combined with the hemorrhagic edema and emphysema typical of the *Bacterium pneumosintes* and the influenzal active agent.

Cultures from the consolidated areas yielded profuse growths of pneumococci or *Bacillus pfeifferi*. Control rabbits injected with the cultivable bodies alone showed only the hemorrhagic edema and the emphysema, without consolidation (or polymorphonuclear cell invasion). Control rabbits injected with the ordinary bacteria, in the small doses employed, showed only a transient polymorphonuclear leucocytosis and no visible lung lesions.

These experiments, therefore, gave results similar to those observed after the intratracheal injection of the active influenzal agent and *Bacillus pfeifferi* or a pneumococcus.

The intratracheal injection of both organisms imitated, in a manner, the probable mode of infection in man. In a second series of experiments, as in the former experiments with the influenzal agent, the intravenous route for the injection of the ordinary bacteria was chosen as a more severe test of concomitant action, even though the blood stream may not be the portal of entry of the lungs in postinfluenzal pneumonias in man.

A series of rabbits was injected intratracheally with a third generation culture of the cultivable bodies, originally derived from the nasopharyngeal secretions of a case in the first epidemic (1918-19). 24 hours later they were given, by ear vein, small doses of a Type IV pneumococcus or of Pfeiffer's bacillus which proved subinfective for control animals.

Under the conditions of the experiment the ordinary bacteria showed a selective localization in the lung tissues where they set up an active infection resulting in more or less extensive lobar or bronchial consolidation, with profuse polymorphonuclear exudation and fibrin formation with necrosis of the vessel walls and thrombus production. The rest of the lung tissue showed the hemorrhagic edema and the emphysema characteristic of infection with *Bacterium pneumosintes* alone. Type IV pneumococci or Pfeiffer's bacilli were recovered from the consolidated areas.

These observations on the results of experimental concurrent infection with the cultivable bodies and ordinary bacteria closely parallel those already described[4] as the result of similar experiments with the active agent and the corresponding ordinary bacteria.

They demonstrate that *Bacterium pneumosintes* possesses the same peculiar property of lowering the threshold of resistance of the pulmonary structure to infection with ordinary bacteria. As a result subinfective doses of Type IV pneumococci and of Pfeiffer's bacilli, for example, become infective and invade the vulnerable tissue, with the establishment of such reactions as are typical of postinfluenzal pneumonia in man. Thus additional proof is afforded of the identity of the active agent of the nasopharyngeal secretions in influenza and the cultivable bodies derived from the same source, and a further parallel is drawn between the accidental or experimental production of concurrent or secondary bacterial pneumonias in animals and the frequent occurrence of similar postinfluenzal pneumonias in man.

SUMMARY.

During the course of animal experiments with the anaerobic filter-passing organisms cultivated from epidemic influenzal sources, certain pulmonary infections with ordinary bacteria have been observed. The experiments also have shown that the lungs of animals infected with *Bacterium pneumosintes* are less resistant than normal lungs to infection with ordinary bacteria. The demonstration of this fact invites a comparison of the course of these experimental bacterial infections with the sequence of postinfluenzal pneumonias attributable to similar organisms in man.

These observations furnish additional proof of the identity of *Bacterium pneumosintes* and the active agent derived from the nasopharyngeal secretions of patients in the early hours of epidemic influenza.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Microscopic lesions in the lungs of a rabbit described in Protocol 2, injected intratracheally with *B. pneumosintes* followed by similar inoculation in 24 hours with *B. pfeifferi*. The diffuse polymorphonuclear exudation is noteworthy. Compare with Fig. 2. × 95.

PLATE 2.

FIG. 2. Microscopic lesions in the lungs of a rabbit described in Protocol 2, and injected intratracheally with the cultivable bodies alone. The hemorrhagic edema and emphysema are noteworthy. × 95.

PLATE 3.

FIG. 3. Gross lesions in the lungs of a rabbit injected intratracheally with the cultivable bodies, and 24 hours later, intravenously with pneumococci. The hemorrhagic consolidation of the left lung, and the hemorrhagic edema and emphysema of the right are noteworthy. Natural size.

FIG. 4. Gross lesions in the lungs of a rabbit injected intratracheally with the cultivable bodies, and 24 hours later, intravenously with *B. pfeifferi*. The hemorrhagic and patchy consolidation of the left lung, and the hemorrhagic edema and emphysema of the right are shown. Natural size.

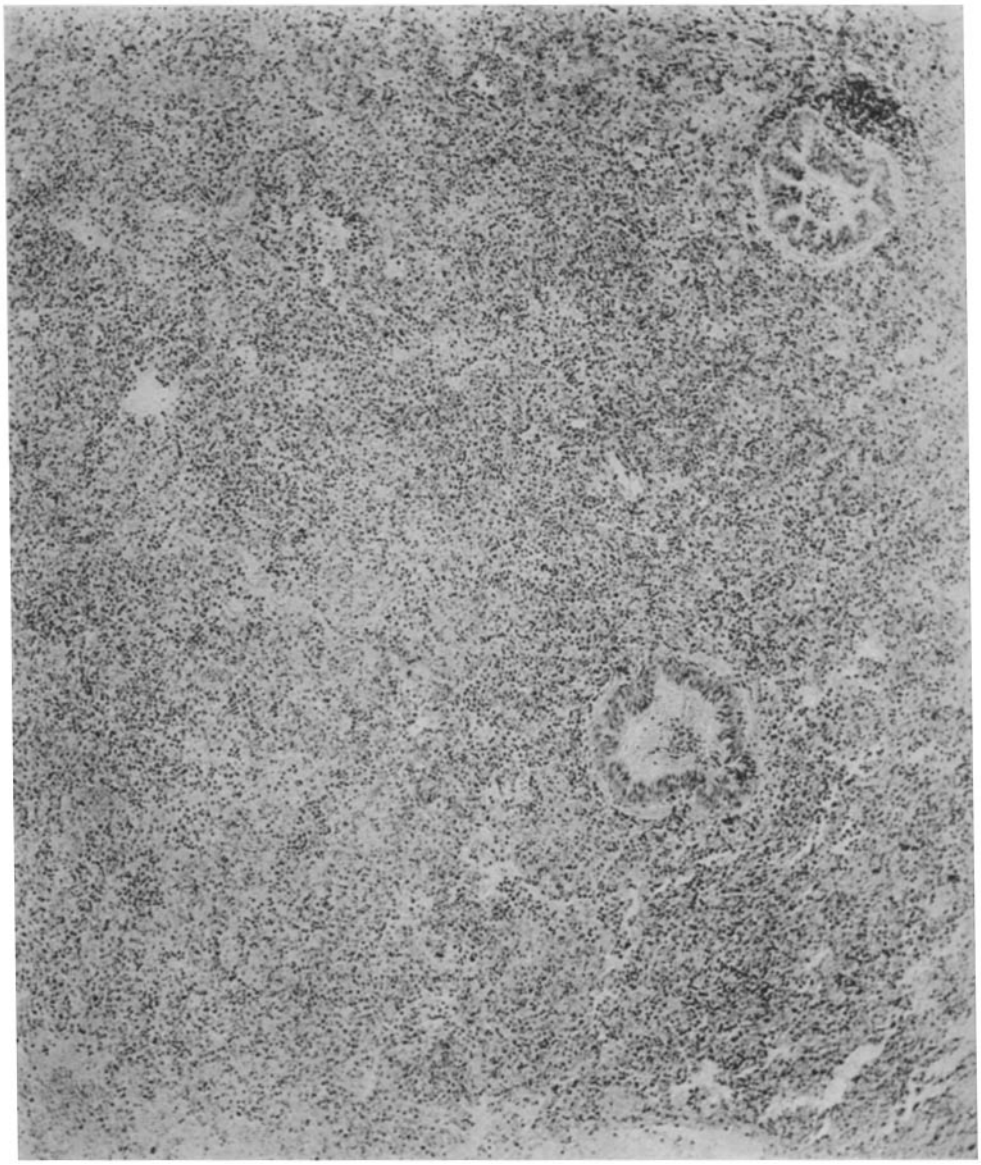


FIG. 1.

(Olitsky and Gates: Nasopharyngeal secretions from influenza. V.)

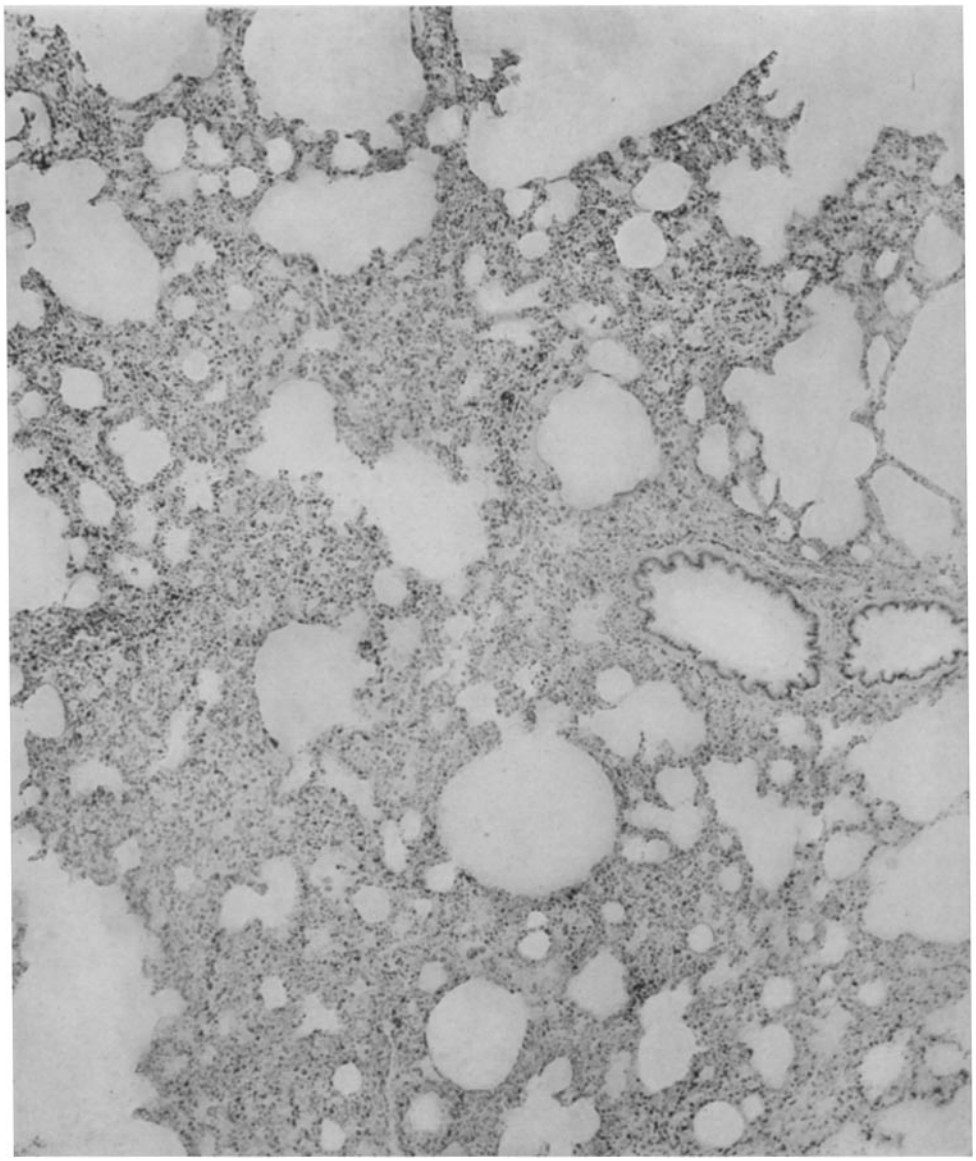


FIG. 2.

(Olitsky and Gates: Nasopharyngeal secretions from influenza. V.)

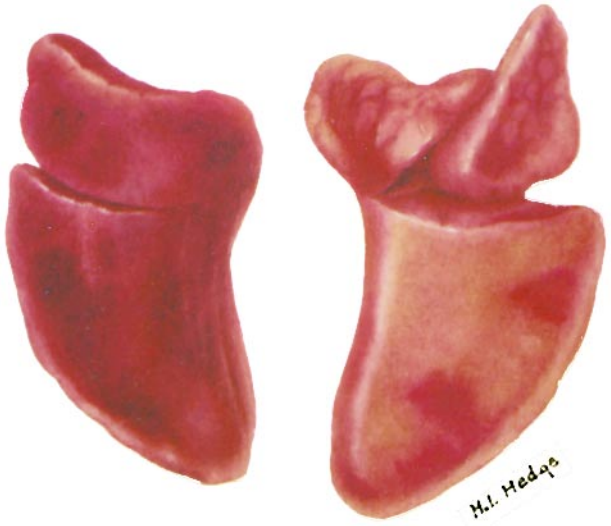


FIG. 3.

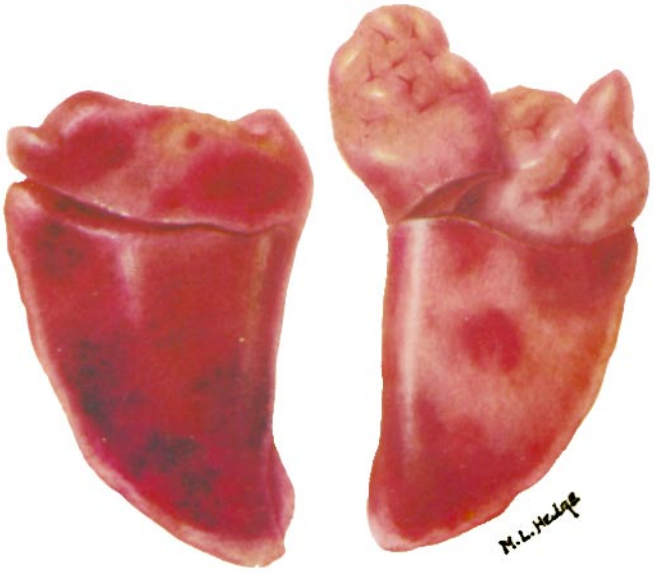


FIG. 4.