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## THE RELATION OF THE BORDET–GENGOU BACILLUS TO THE LESION OF PERTUSSIS.*

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Introduction. — We have already shown† that the primary essential lesion of whooping cough consists in the presence of masses of minute bacilli between the cilia of the epithelial cells lining the trachea and bronchi. Their action is chiefly mechanical: they interfere with the normal movements of the cilia by sticking them together; in this way the microörganisms furnish a continual irritation which results in the symptoms peculiar to the disease.

Experimental Production of Lesion. — The discovery of the primary and apparently characteristic lesion of whooping cough naturally suggested an attempt to determine whether or not the organism growing between the cilia corresponded to the bacillus described by Bordet and Gengou as the cause of whooping cough. Klimenko's demonstration that it was possible to infect puppies with that bacillus afforded a simple means of testing the matter.

Our first experiment was to find out if we could produce in animals the same peculiar lesion that we had found in man. For this purpose a puppy was inoculated intratracheally at intervals of one week for five weeks with sputum collected from patients in the acute stage of whooping cough. The animal was killed at the end of six weeks

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†Mallory and Hornor, Journal Medical Research, 1912, xxvii, 115–123.

and the tissues fixed in Zenker's fluid. Paraffin sections stained in the way already described showed numerous minute bacilli between the cilia of many of the epithelial cells lining the trachea. They corresponded morphologically in every way with those occurring in the human tissues.

Pure Cultures. — The next step was to obtain if possible a pure culture from a case of whooping cough and repeat the experiment with it.

Sputum was examined from thirty patients in the acute stage of the disease. The characteristic bacillus was found in all in large numbers and often in an almost pure state both free and enclosed in polymorphonuclear leucocytes and rarely between the cilia of desquamated epithelial cells. After repeated failures, pure cultures were finally obtained directly from the sputum from two different patients. A third pure culture was obtained indirectly from a puppy infected by means of sputum injected into the trachea. No attempt was made to obtain a large variety of cultures for study and comparison as work of this character had already been done by Bordet and Gengou and repeated by Klimenko.

It was found impossible to start cultures except on the potato-blood-agar medium devised by Bordet and Gengou. For the first twenty-four hours the growth is slight even on this material. After that length of time, however, the bacilli grow readily and abundantly, and later may be transferred to various other media such as blood serum, and especially veal agar and veal bouillon. The single colonies on solid media may reach a diameter of two to four millimeters in forty-eight hours. They are rather thick and almost colorless and transparent at first; later they are slightly grayish and more or less opaque. The colonies possess a tenacious mucoid character which becomes more distinct in older cultures, so that a whole colony may be lifted up at once on a platinum needle. It is probably owing to this peculiarity that the organisms adhere to the cilia of the cells and stick or mat them together.

For comparison and control we used a culture of the

original Bordet-Gengou strain, for which we are indebted to the courtesy of the Parke-Davis Company.

Morphology of Bacillus Pertussis. — In cover-slip preparations made from sputum the microörganisms appear as minute bacilli with rounded ends. They are of about the size of influenza bacilli or a little larger and more regularly ovoid. They are Gram-negative and stain lightly with the usual basic anilin dyes. In smears from cultures on potato-blood-agar and on blood serum the bacilli are so short as to resemble for the most part micrococci. On some of the other media, such as veal agar, they tend to grow longer and occasionally present large forms. If the organism is replanted on the potato-blood-agar medium the large forms disappear.

Experimental Work with Pure Cultures. — Several puppies were directly inoculated in the trachea with pure cultures. In two of the puppies the success of the inoculation was readily demonstrable in sections. The bacillus was reobtained in pure culture from one of the dogs, but not from the other. In two puppies the results were negative and also in two others inoculated with the Parke-Davis culture. These four dogs in which the results were negative all came from one litter and were strong, healthy animals. The organisms were obtained in cultures, however, from the trachea or lung in each instance. Judging from other experiments the bacilli were probably present between the cilia in very small numbers, but none happened to be present in the sections cut and stained.

Three adult monkeys (Rhesus macacus), inoculated intratracheally, showed no symptoms, and were therefore not killed. Histological examination might have shown the characteristic lesion. Klimenko obtained fairly well marked symptoms with several monkeys, but other experimenters have failed of success.

He states that he failed to get symptoms when he inoculated young rabbits and guinea-pigs. Infection readily takes

place, however, as is shown by the presence of the characteristic lesion (see Fig. 2, Plate XXVII.) in the first rabbit we inoculated. The experiment was repeated with four others in order to see if any symptoms could be detected. They were inoculated on the first, third, fourth, and fifth days by dropping a small amount of bouillon culture into the nares. Three of the animals became more or less emaciated and all had fairly free secretion from the nares, but they developed no other symptoms. They were killed at the end of six, six, fifteen, and twenty-five days respectively. The infecting organism was recovered in pure culture from the first three, but the original cultures from the fourth rabbit were too greatly contaminated to render this feasible.

In the animals successfully inoculated the bacillus pertussis was found regularly in the trachea, less often in the larger and smaller bronchi, and in four instances (all rabbits) in the nares. In all of these situations it occurred between the cilia of the lining epithelial cells. In addition it was occasionally found free in the secretion in the trachea or bronchi.

Infection by Contagion. — Several puppies kept in the same cage with the first one inoculated with sputum acquired infection by direct contact or by contagion and showed the typical lesion in the trachea and lungs. This result confirms the observation of Klimenko, that normal puppies are readily infected if kept in more or less intimate contact with inoculated animals.

Symptoms of Inoculated Animals. — The chief symptoms presented by puppies are sneezing and coughing, which appear in from two to six days. There is usually also more or less secretion from the nose and eyes. In a few days the coughing becomes more severe and is spasmodic in character. According to Klimenko, who made a special study of the clinical phenomena following inoculation, the puppies almost always die in from two to six weeks.

The only symptom shown by the rabbits was emaciation. No coughing or sneezing was ever observed.

Inoculation of Animals. — Various methods of inoculating animals in the respiratory tract with the pertussis bacillus were tried: through a tracheotomy wound; by the introduction of a swab, which had been dipped into a veal bouillon culture, directly into the nares and trachea; by injecting a culture into the trachea through a small catheter. In the end the following method was found to be much the simplest procedure: hold the animal on its back with its mouth shut and with its nose pointing upwards. From a glass syringe or tube let a veal bouillon culture flow a drop at a time into the nares. More or less of the material is sure to be inhaled. Inoculation by this method can be repeated daily if desired. All inoculations by this method were successful. On this account a culture of the strain obtained originally from Bordet was tried again on a young rabbit. A growth in veal bouillon was dropped into the nares on four successive days and the animal was killed on the eighth day. The bacillus was reobtained in pure cultures from the trachea and bronchi and was found microscopically in moderate numbers in sections of the nares, trachea, and bronchi (Plate XXVIII., Figs. 6 and 7).

Lesion in Infected Animals. — The lesion in the infected animals corresponds in every way with that in man, but the number of organisms is rarely so great. The bacilli are not as a rule uniformly distributed over the lining epithelium. They are few or numerous in certain areas and wholly lacking in others. Occasionally only single cells or small groups of them have any organisms among the cilia.

The animal lesions show clearly that the cilia are not destroyed, and that the cells are not in any noticeable way injured. On the other hand, a certain number of polymorphonuclear leucocytes can usually be found migrating between the epithelial cells to reach the free surface. Occasionally they are fairly numerous. Only a moderate accumulation of lymphocytes in the wall of the trachea was observed; the blood was not examined for lymphocytosis.

## SUMMARY AND CONCLUSIONS.

The failure up to the present time to observe the bacillus of whooping cough in its peculiar and characteristic location in the respiratory tract is probably due to its small size and to its staining but faintly by ordinary methods.

The reason the Bordet-Gengou bacillus has not been unqualifiedly accepted as the cause of whooping cough is largely due to its presence having been demonstrated only in connection with the disease, not in connection with any evident lesion. It might have been a secondary invader like the streptococcus in scarlet fever. We have demonstrated the primary essential lesion of the disease and the characteristic relation of the causal agent to it. In addition we have been able, with sputum and with pure cultures of a microörganism corresponding in every way with the Bordet-Gengou bacillus, to produce the same characteristic lesion in young animals and in four instances (one puppy and three young rabbits) to obtain the organism again in pure culture. Moreover, we have produced the same lesion in the nares, trachea, and bronchi of a young rabbit after infecting it through the nares with a subculture of the bacillus obtained originally from Bordet and were able to grow the microörganism again in pure culture. We have, therefore, supplied the steps which have heretofore been lacking, according to Koch's laws, for the complete demonstration that the Bordet-Gengou bacillus is the cause of whooping cough. This result should encourage further investigation in search for a vaccine or antitoxin which may be of use in the treatment of the disease. The diminution in numbers and final disappearance of the microörganisms under ordinary conditions in the course of comparatively few weeks strongly suggests that conditions for growth become unfavorable, probably owing to the development of some antibody.

## DESCRIPTION OF PLATES XXVII. AND XXVIII.

FIG. 1. — Section of trachea from puppy inoculated through a tracheotomy opening with sputum from acute cases of whooping cough. Few to many minute bacilli are present between the cilia. A polymorphonuclear leucocyte on the surface contains several of them. x 1,500.

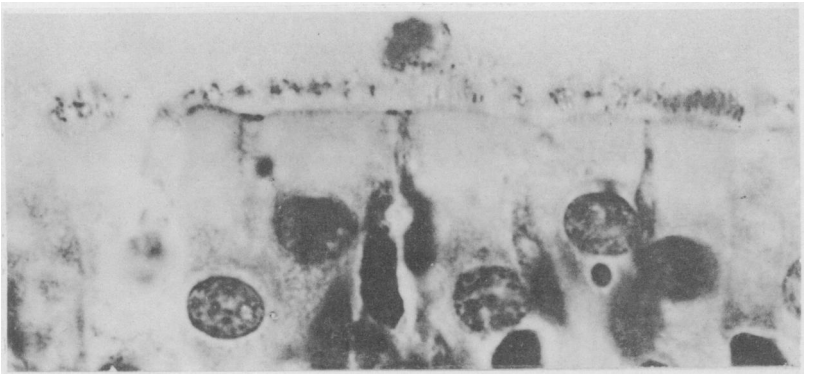
FIG. 2. — A section of the trachea from a young rabbit inoculated with sputum in the same way. The bacilli are fairly numerous between the cilia. x 1,500.

FIG. 3. — A section of the trachea from a puppy inoculated intratracheally with a pure culture of a bacillus corresponding in every way with that described by Bordet and Gengou as the cause of whooping cough. x 1,500.

FIG. 4. — Section of the trachea from a puppy inoculated intratracheally with a pure culture of another strain of microörganism identical morphologically and culturally with the first. The bacilli are numerous between the cilia and also in the secretion on the surface. x 1,500.

FIG. 5. — Section of nasal mucous membrane from a young rabbit inoculated with a pure culture through the nares on four separate days and killed at the end of one week. The bacilli are numerous between the cilia. x 1,500.

FIGS. 6 and 7. — Sections of the trachea from a young rabbit inoculated through the nares with a sub-culture of bacillus pertussis obtained originally from Bordet. The microörganisms are very numerous at the points where they have been able to penetrate between the cilia and multiply. x 1,500.



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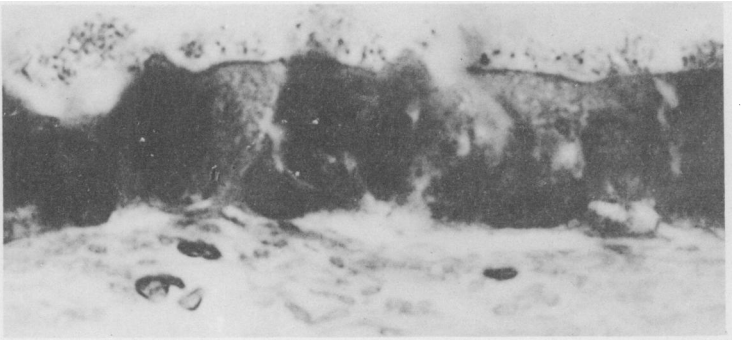

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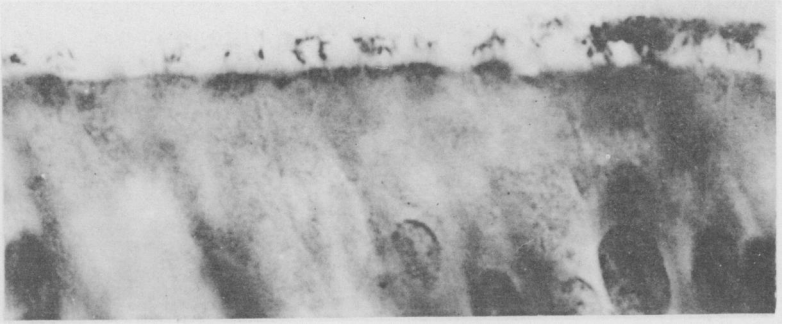

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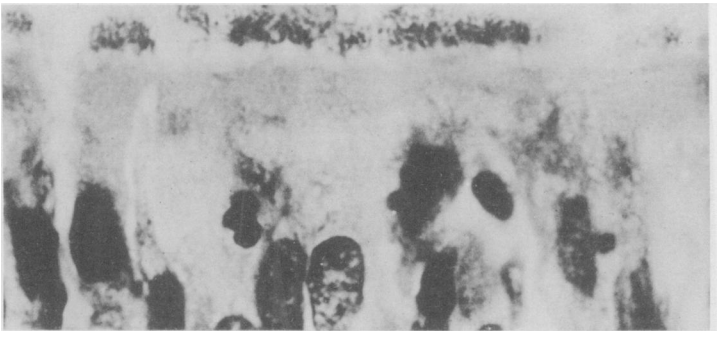

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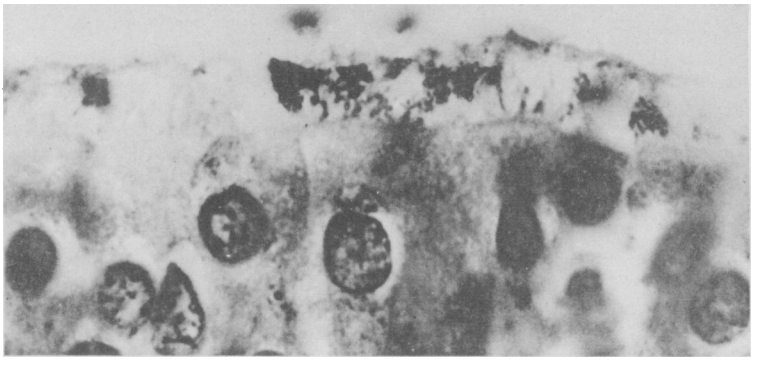

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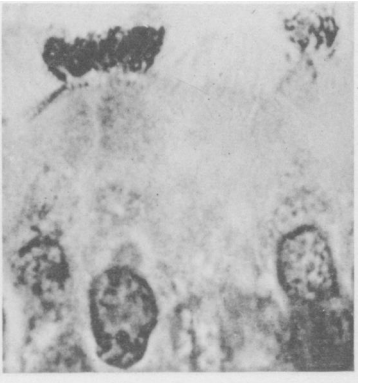

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