INTERSTITIAL BRONCHOPNEUMONIA

II. Production of Interstitial Mononuclear Pneumonia by the Bordet-Gengou Bacillus*

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Plate 18

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The rôle of the Bordet-Gengou bacillus as the primary etiologic agent of pertussis has been questioned by McCordock (1) and Rich (2). The latter worker reviews the literature on the subject and draws attention to the fact that the criterion of the successful production of pertussis in animals has often been the presence of a paroxysmal cough or "whoop." This "whoop," he believes, can be caused by other types of bacteria if sufficient numbers are present in the trachea and bronchi. This idea is supported by the work of Blake and Cecil (3) who state that monkeys injected intratracheally with Pfeiffer's bacillus have at times a severe racking cough. Furthermore Brown (4) describes an instance of a child infected with *Bacillus bronchisepticus* who had typical symptoms of pertussis.

Certain similarities between pertussis and measles or other virus diseases led McCordock (1) and Rich (2) to suggest that pertussis may be a virus infection. Rich also suggests the permanent immunity following recovery, the extreme infectivity of the causative agent of pertussis, and the occurrence of encephalitis as an occasional complication are evidences in favor of the idea that whooping cough is caused by a virus. Both investigators cite as additional evidence of the virus nature of the causative agent of the disease the presence of intranuclear inclusions and interstitial mononuclear pneumonia in the lungs of children dying of pertussis.

In spite of the observations mentioned above direct evidence that a virus is the primary etiologic agent of pertussis is still lacking. More recently Rich, Long, and their associates (5) failed to produce symptoms suggestive of whooping cough in chimpanzees by inoculation of filtered nasal washings from a case of

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pertussis, but were successful in infecting chimpanzees by means of unfiltered nasal washings and pure cultures of the Bordet-Gengou bacillus. MacDonald and MacDonald (6) also failed to produce pertussis in children with filtrates but succeeded with pure cultures of the bacillus. Culotta and his associates (7) succeeded in infecting monkeys with the organism, but neither filtered nor unfiltered nasal washings of children with pertussis proved to be infectious.

In a previous paper (8) we showed that certain bacterial toxins are capable of producing an interstitial mononuclear pneumonia similar to that caused by the viruses. It was pointed out in that communication that the toxins which caused this type of pneumonia had in common with the viruses certain properties; *e.g.*, the ability to produce a prolonged or a permanent immunity. This led us to the study of the lesions which could be produced in the lungs of rabbits by the Bordet-Gengou bacilli and the typhoid bacilli, both of which organisms, like viruses and toxins, possess strong antigenic properties.

The work described in this paper was designed to show that the Bordet-Gengou bacillus of itself can produce an interstitial mononuclear pneumonia and that the occurrence of this type of reaction in the lungs cannot be used to support the idea that pertussis is a virus disease.

Methods and Materials

The animals used in the experiments were young adult rabbits which had been tested for the presence or absence of *Bacterium lepisepticum* and *Bacillus bronchisepticus* by the method described in the first paper of this series. All rabbits harboring these organisms were excluded.

Medium.—The medium for the cultivation of the pertussis bacillus was similar to that described by Bordet and Gengou (9) with the exception that 25 per cent defibrinated human blood was substituted for the horse blood.

Organisms.—The strain of Bordet-Gengou bacillus used in this work was obtained from Dr. W. A. Jameson of the Eli Lilly Co. and the virulence of the organism was maintained by growing it on the above described medium. A virulent strain of *Bacillus typhosus* which had been kept on beef infusion agar slants since its isolation in 1918 was employed.

Lysates and Vaccines.—Various methods were used in preparing toxic extracts from the Bordet-Gengou bacillus. The bacteria obtained from the 72 hour growth on 30 Bordet-Gengou slants were scraped off into 10 cc. of sterile distilled water. The suspension was then frozen and thawed 6 times. No disintegration of the bacilli was found in a stained smear. The supernatant fluid and the sediment, both of which were sterile, were kept for animal inoculation. A second lysate

was made by the method described by Teissier and his associates (10). The organisms were powdered by grinding with sterile salt in a mortar. The powder was then suspended in enough distilled water to make the suspension isotonic after which it was kept at room temperature for 24 hours. The supernatant fluid was sterile and clear. This fluid was kept for animal inoculation. Commercial vaccines prepared by Eli Lilly and Co. according to the Krueger and the Sauer techniques were employed.

Dose.—In every instance the total volume of the material injected into each animal was 1 cc. The pertussis organism was grown for 48 hours on 1 or 2 Bordet-Gengou agar slants and then suspended in sterile normal saline. The number of organisms obtained in this manner varied between 50 and 100 billion. The typhoid bacilli were grown on beef infusion agar slants. The 18 hour surface growths of 2 slants suspended in 10 cc. of saline were used. After several passages through the rabbits' lungs, an extensive lesion could consistently be obtained by use of the growth from ¼ of an agar slant.

Inoculation.—The inoculations were made intratracheally as described in the first paper of this series.

Necropsy.—The necropsies were done as described in the preceding paper except that, in addition to the routine cultures of the lungs, the bronchi and lungs were tested for the presence of the Bordet-Gengou bacillus by means of culture on Bordet-Gengou plates.

Fixation and Stains.—After the lungs were removed from the body they were inflated with air and fixed in Zenker's solution (Helly's modification). After fixation the lungs were embedded in paraffin. Sections were stained with hematoxylin and eosin, Mallory's eosin-methylene blue, MacCallum's bacterial stain, and by the method described by Brown and Brenn for staining Gram-negative bacilli.

EXPERIMENTAL

The experiments were designed to study the effect of living Bordet-Gengou organisms and lysates and vaccines of these bacteria on the lungs of rabbits. Similar experiments were performed with *Bacillus typhosus*. The latter organism was chosen because typhoid may be followed by an encephalitis and permanent immunity similar to those following pertussis. Although we were not able to find reports of good morbid anatomical studies of the encephalitis following typhoid fever, the clinical histories reported by Wieland (11), Hillemand and his associates (12), and others indicate that this encephalitis is similar to that following pertussis.

Experiment 1.—22 rabbits were used in the experiment and each received the organisms from 1 or 2 Bordet-Gengou agar slants suspended in 1 cc. of saline.

The animals were killed at intervals of from 12 hours to 2 weeks after inoculation. The majority, however, were killed at 72 and 96 hours.

Morbid Anatomy. Gross.—The lungs from the rabbits killed at the end of 12 and 24 hours show little except some congestion and a few areas of hemorrhage. After 72 hours there is usually an area of consolidation which as a rule is near the hilus of the left lung posteriorly but which at times may involve other portions of the lungs. In some animals such areas are firm and dark purple, in others necrotic.

Microscopic.—The lungs removed 12 hours after death show a number of polymorphonuclear cells. These are most prevalent around the bronchi but are also in the alveoli, the interstitial tissue, and the perivascular lymphatics. A number of red blood cells and an occasional mononuclear cell are present in a few focal areas.

At the end of 3, 4, and 5 days the picture is essentially similar to that produced with bacterial toxins (8). The animals receiving a large dose as a rule show necrosis of lung tissue while those receiving a small dose show evidence of proliferation. The proliferative lesion will be described first. Some of the alveoli are lined with cells having vesicular nuclei, small nucleoli, and scanty, poorly stained cytoplasm. Some of these cells are undergoing mitosis but mitotic figures are not as numerous as in the case of tissues injured by bacterial toxins. The lumina of the alveoli, both those with proliferating lining cells and those without, are filled with mononuclear cells (Fig. 1). Such cells are similar to those attached to the walls except that their cytoplasm is more plentiful. They resemble monocytes and macrophages. Intermixed with these cells are polymorphonuclear cells in various stages of degeneration. In some instances there is rather extensive hemorrhage. All of the perivascular lymphatics are filled with lymphocytes and in many instances the walls of the blood vessels are infiltrated with them. In no instance, however, is a thrombus found. There is a definite increase in lymphocytes around the bronchi and bronchioles. The mucosa of the bronchi and bronchioles is infiltrated with polymorphonuclear cells, and occasionally there is a hyperplasia of the lining epithelium.

The animals which received the large dose show infarct-like areas of necrosis. This necrosis, however, is not due to thrombosis. Although more extensive involvement of the blood vessels is seen than described above, no thrombi are observed. That the necrosis followed the proliferation is evidenced by the fact that the alveolar walls are thickened even in the necrotic areas. The areas of necrosis are infiltrated in some instances with polymorphonuclear cells, but this is less extensive than in the toxin experiment previously reported (8). In other portions of the lungs rather extensive hemorrhage is seen. After 1 and 2 weeks the picture is more or less similar to that already described except that the proliferation had become more marked. At first sight the number of bronchioles seem to be increased throughout the lung. On closer inspection these are seen to be alveoli lined with cells which might be mistaken for cells lining the bronchioles (Figs. 2 and 3). There are still some polymorphonuclear cells present, but they are not as numerous as in the earlier lesions. The perivascular lym-

phatics are still filled with lymphocytes. The proliferation of the cells lining the alveoli has in some instances proceeded to such an extent that they form a syncytium of cells. There are a few multinuclear or giant cells similar to those found in the toxin experiment. The lymphoid tissue around the bronchi is still increased. Only a slight amount of fibrosis is found and it is thought that the structure of the lungs would return to normal if sufficient time were allowed.

In none of the rabbits' lungs were inclusion bodies found. The Bordet-Gengou bacilli were only recovered in animals killed within 48 hours after inoculation. No other organisms were found in the lung by means of stains or cultures.

Experiment 2.—18 rabbits were inoculated intratracheally with the various lysates and vaccines. The animals were killed 72 and 96 hours after inoculation.

Morbid Anatomy.—In the gross the rabbits' lungs show only a slight reddening. Microscopically a few areas of mononuclear cells are found.

Experiment 3.—8 rabbits were injected intratracheally with a suspension of the typhoid bacilli. The first rabbit was inoculated with the material from 2 agar slants. A small lesion resulted and a large number of typhoid bacilli were cultured from it. After passage of the bacilli through several animals the inoculum had to be reduced from 2 agar slants to ¼ of one agar slant as a larger dose caused death of the animals in less than 24 hours. The animals which received bacilli from ¼ agar slant had lesions about the same size as those which received the pertussis organisms. Typhoid bacilli did not grow out on solid media smeared with the lung tissue. However, in some instances the bacilli could be recovered from broth tubes inoculated with a large piece of lung.

Morbid Anatomy.—The lungs of the rabbits inoculated with typhoid bacilli resemble in every respect those infected with pertussis bacilli described in Experiment 1.

Controls.—In order to be certain that the reactions described in the lungs were due to the organisms injected and not to a latent virus or to extraneous materials which may have been washed from the slants, the following control experiments were conducted. In all instances the animals were killed 72 hours after inoculation.

Medium.—2 rabbits were inoculated with material prepared in the following manner. 2 cc. of sterile normal saline were added to an uninoculated Bordet-Gengou slant. The slant was scraped with the inoculating loop so that a blood-tinged cloudy suspension was obtained. No lesions were produced by intratracheal inoculation of this material.

Avirulent Organisms.—6 rabbits were inoculated with avirulent Bordet-Gengou organisms. No lesions with the exception of a few small hemorrhages were found in the lungs of these rabbits.

Virus Control.—A rabbit's lung with a typical lesion was triturated with an abrasive. A 10 per cent suspension was made, and 1 cc. of this was injected into

each of 3 rabbits. The lungs of these rabbits show nothing more than small hemorrhagic areas.

DISCUSSION

The morbid anatomical changes described at this time are similar to those previously described as resulting from the action of bacterial toxins (8). Through the courtesy of Drs. Rivers and Francis we had the opportunity of comparing our sections with theirs showing lesions produced by the viruses of psittacosis and epidemic influenza. The lesions are similar. A study of the lung lesions found in several cases of pertussis as well as descriptions of the pneumonia in pertussis by McCordock (1) and Rich (2) led us to believe that the lesions in the lungs of rabbits inoculated with pertussis bacilli could not be differentiated from those found in human beings with pertussis. On the basis of these observations we feel justified in maintaining that the presence of an interstitial mononuclear pneumonia in pertussis is not evidence that pertussis is a virus disease.

The similarity of the pneumonia caused by pertussis bacilli to that produced by bacterial toxins led us to try to determine whether a toxin capable of producing such a lesion could be obtained from the Bordet-Gengou bacillus. We have not yet been able to derive a toxin from the organism *in vitro* and our experiments with the lysates and vaccines are not at all conclusive in this regard. Nevertheless the inability to recover viable Bordet-Gengou bacilli 48 hours after inoculation, the ease with which typhoid bacilli were recovered from the lungs of rabbits which had slight lesions, and the difficulty in recovering them from good lesions suggest that the reactions are due to some substances liberated by the death of the organism in the animal body. It is interesting to note that Bordet and Gengou (13) also failed to recover the pertussis organism from the peritoneum of guinea pigs inoculated with a fatal dose.

The possibility that the lysates and the vaccines retain their immunizing properties in spite of losing their toxic action is being investigated. The fact that an interstitial mononuclear pneumonia can be produced in rabbits with pure cultures of the Bordet-Gengou bacillus makes possible such an investigation.

Because of certain similarities between the Bordet-Gengou bacillus,

Bacillus bronchisepticus and *Bacterium lepisepticum,* and since many rabbits harbor the last two organisms mentioned, the results of much of the previous work on pertussis in animals have been questioned. The presence of these organisms in our experiments was carefully avoided, inasmuch as the rabbits' nares were cultured before the experiments were started, and the lungs at necropsy were cultured and stained in order to demonstrate their absence. Whenever these organisms were encountered the rabbits were discarded, and we believe that errors from this source have been obviated.

In experimental work of this nature one must consider the possibility of the stimulation by the experimental procedures of a latent virus in the animal body. In our work, however, the excitation of a latent virus can be excluded by the absence of inclusion bodies in the lungs and by the inability of the material taken from typical lesions to incite similar lesions in other rabbits.

McCordock (1) and Rich (2) questioned the relation of the inclusions in the lungs to the etiologic agent of pertussis. The failure to find inclusions in our experimental animals as well as the fact that Von Glahn and Pappenheimer (14) have described similar inclusions in the lungs of an adult not affected with pertussis lead us to believe that the intranuclear inclusions found in patients dying of pertussis bear no etiologic relation to the disease.

The more or less permanent immunity induced by an attack of pertussis and the encephalitis that occasionally follows the disease are cited by Rich (2) as reasons for believing that pertussis is a virus infection. We have already indicated that both of these phenomena are associated with typhoid fever. Hence it is not unreasonable to suppose that a bacterium instead of a virus may be the cause of whooping cough.

CONCLUSIONS

Pure cultures of Bordet-Gengou bacilli produce in rabbits an interstitial mononuclear pneumonia which cannot be differentiated from that occurring in children dying of pertussis or from that caused in animals by the viruses of epidemic influenza and psittacosis. A similar pneumonia can be produced in rabbits by typhoid bacilli.

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EXPLANATION OF PLATE 18

FIG. 1. Section of rabbit's lung 96 hours after intratracheal inoculation of Bordet-Gengou bacilli. There is a marked increase of the interstitial cells and a proliferation of the cells lining the alveoli. ×210.

FIG. 2. Section of rabbit's lung, killed 7 days after intratracheal inoculation of Bordet-Gengou bacilli, showing alveoli lined with proliferating cells so that the alveoli resemble a bronchiole. There is also an increase in the interstitial tissue. ×450.

FIG. 3. Section of rabbit's lung, killed 7 days after inoculation of Bordet-Gengou bacilli. Note the mononuclear cells and also the proliferation of the lining cells of the alveolus. ×830.