Some Observations on Diagnosis of *Bordetella bronchiseptica* Infection in Guinea Pigs

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INTRODUCTION

Bordetella bronchiseptica (*B. bronchiseptica*) has been isolated from the respiratory tract of many kinds of wild, domestic and laboratory animals, such as the rat[2], guinea pig, rabbit, ferret, cat, monkey[12], dog[4], pig[15], turkey [5], and horse[7], and sometimes caused pneumonia and rhinitis. Man could also occasionally become infected[12,13,17]. In guinea pigs, it is generally regarded that the organism shows a low degree of virulence, in spite of a high degree of infectivity. Beer [1] stated that *B. bronchiseptica* played only a secondary role in infectious diseases. Ganaway et al.[8] and Woode and McLeod [18], however, reported fatal epizootics in their breeding colonies. Thus, it was considered that the initiation of the epizootic was provoked by some form of stress such as malnutrition, poor ventilation, cold temperature, etc. [11,16]. An extensive review of earlier investigations and a detailed description of the organism were given by Winsser [17].

Diagnosis of *B. bronchiseptica* infection in guinea pigs is usually made by post-mortem bacterial examination of the trachea and lung, since the affected animals rarely show any clinical signs characteristic to the infection, except usual symptoms of respiratory infection, less gain of weight or death. Horie et al.[10] stated that extensive bronchopneumonia caused by *B. bronchiseptica* was frequently found at post-mortem examination of animals healthy in appearance. On the other hand, Woode and

McLeod [18] successfully used nasal swabbing method for detection of infected animals, but Ganaway et al.[8] failed to demonstrate carriers by examination of the external nares and described that the carrier state could be detected accurately only at necropsy.

In 1960, Horie et al.[10] indicated that *B. bronchiseptica* was prevailing among guinea pigs in an animal house of our institute, which were purchased weekly from some registered commercial breeders and used for the assay of biological products and other experimental purposes. The affected animals showed various degrees of bronchopneumonia at autopsy which occasionally led them into poor growth, but fatal cases were very rare. Since then, an extensive survey of the organism was performed on guinea pigs in the animal house and in breeding colonies of the breeders, to distinguish contaminated colonies from uncontaminated ones and to use animals free from the organism in routine works. The purposes of the present paper are to describe bacteriological, pathological and serological features of the infection and to present diagnostic methods studied in the survey.

MATERIALS AND METHODS

Guinea pigs : Guinea pigs of Hartley strain were mainly collected from 4 separated breeding colonies being contaminated with *B. bronchiseptica* and from the animal house of our institute, in which hundreds animals were introduced weekly from 21 registered breeding colonies including the above four. In serological observa-

tions, additional guinea pigs were obtained as negative control from seven uncontaminated colonies which were proved to be free from the organism for longer than five years by repeated examinations. In all the breeding colonies, polygamous system was adopted, making a group of three to seven females and one male. In our animal house, five adults were usually maintained in a cage during the use. Guinea pigs in our animal house and 2 contaminated breeding colonies were fed with pellet, water and sometimes green food, but those in other colonies with bran and green food.

Isolation and identification of the organism : Samples were obtained by cotton swabbing or scraping with a loop for the mucus membrane and by scarifying a piece of material with scissors for the parenchymal organs. They were cultured on one or all of 5 per cent horse blood agar, modified Endo agar or MacConkey agar for 48 hours at 37°C. The modified Endo agar was prepared by exchanging lactose in Endo agar for glucose, because *B. bronchiseptica*, carbohydrate non-fermenter, could be distinguished easily from other contaminants. Developed colonies were identified as *B. bronchiseptica* by the following properties : slow colony formation on nutrient, blood or MacConkey agar, uniform growth in broth, Gram-negative staining, positive motility, failure to ferment carbohydrate but alkaline production in Barsiekow's glucose medium, utilization of citrate as a sole source of carbon and splitting of urea. In case of need, slide agglutination test was performed using a rabbit antiserum against a typical strain of the species.

Pathological examination : Pathological examination was made on only the lung. After a small portion of tissue was taken aseptically for bacteriological examination from a most extensively affected area or from the intermediate lobe, when no lesion appeared, the lung was fixed by 10 per cent formalin. Histopathological examination was performed on paraffin sections being stained with Mayer's hematoxylin and eosin, periodic acid-Shiff and Azan stains.

Antigens for serological test : Strain 154N,

originated from the nose of a naturally infected guinea pig, was mainly used in this study. Typical and smooth colonies were selected from growth on nutrient agar, though rough variants were rarely seen, and used for antigen preparations. Antigens were 1) live cultures grown in or on trypticase soy broth (TSB), nutrient broth (NB), peptone water (PW) and trypticase soy agar (TSA), 2) formalin killed TSB and TSA cultures, 3) TSB cultures, heated at 60°C, 100°C and 120°C for one hour and 4) TSA culture treated with alcohol. The alcohol treatment was performed by the following methods. A cell suspension was made with 96 per cent ethyl alcohol, heated at 60°C for one hour and centrifuged, and then the resulted deposit was suspended in saline. The concentration of all the antigens was adjusted to McFarland's nephelometer no. 3 with trypticase soy broth, except NB and PW cultures for which corresponding medium was used as diluent.

Agglutination test : The tube agglutination test was used throughout this work. Guinea pig sera to be tested were diluted 5 times with saline and inactivated at 56°C for 30 minutes. After two-fold dilutions were made on the inactivated sera using the antigen suspensions mentioned above, resulted serum-antigen mixtures were left at 37°C for 2 hours and then at 4°C overnight.

Absorption test : About 5×10^{10} cells were suspended in 1 ml of serum diluted 10 times with saline. The suspension was left at 37°C for 2 hours, sometimes shaking it vigorously, and then at 4°C overnight. After centrifugation, the resulted supernatant was submitted to agglutination test for checking unabsorbed antibodies.

RESULTS

Bacteriological findings

1. Culture media for isolation of *B. bronchiseptica*

Three different culture media, horse blood agar, modified Endo agar and MacConkey agar, were examined on usefulness for primary isolation of the organism.

These media were first compared on the rate

of growth of the organism, using pure culture of 10 stock strains. Eighteen hour broth cultures of the strains were diluted with saline to contain about 1,000 viable cells per ml and 0.2 ml of the dilutions were plated on the media. Developed colonies were counted after 48 hour incubation period at 37°C. There was no difference between numbers of colony on the blood agar and the modified Endo agar, while roughly half the number of cells grown on the two media formed colonies on MacConkey agar. Among the media, however, no remarkable discrepancy was observed in incubation period required for colony formation and in colony size.

Usefulness of the media was also compared by practical use in isolation of the organism from the nose, trachea and lung of naturally infected guinea pigs. Isolation rate of the organism from the trachea was almost same among the three media but that from the other sites was considerably varied by the culture media used. In nasal samples, the rate was increased by use of MacConkey, modified Endo and blood agars, in that order, indicating 96.8 per cent, 91.9 per cent and 66.1 per cent, respectively. This might be due to the difference of their inhibitory effects to other contaminants, especially Gram-positive bacteria which were predominant in the nose of guinea pigs. On the contrary to this, the lung material yielded the organism more frequently on modified Endo or blood agar than on MacConkey agar. About 20 per cent of positive samples was failed to detect by use of MacConkey agar. On the bases of these findings, nasal samples were cultured on MacConkey agar but the trachea and lung on blood agar in subsequent works.

2. Favored locus of *B. bronchiseptica*

Some experiments were carried out to demonstrate favorite sites of localization of *B. bronchiseptica* in guinea pigs and at the same time, to evaluate the diagnostic usefulness of the external nares which were only places being able to check the organism in living animals. Cultivation was made on the external nares, upper (cervix) and lower (chest) parts of the trachea and the intermediate lobe of the lung of naturally in-

fecting 296 animals. In some of them, the nasal cavity, tonsil, pharynx, larynx, liver, spleen and kidney were also examined.

Of the 296 infected guinea pigs, as many as 239 harbored the organism throughout the respiratory tract or from the external nares to the lower trachea. It was also noteworthy that 47 animals carried the organism only in the nose, upper trachea or both of them. Totalling by individual site, as shown in Table 1, 261 or 88.2 per cent

Table 1. Isolation of *B. bronchiseptica* from several organs of infected guinea pigs

Organs	Positive/Examined	Per cent
External nares	261/296	88.2
Nasal cavity	49/52	94.2
Tonsil	62/118	52.5
Pharynx	28/64	43.8
Larynx	48/64	75.0
Upper trachea	267/296	90.2
Lower trachea	244/296	82.4
Lung	115/296	38.9
Liver	0/106	0
Spleen	0/106	0
Kidney	0/106	0

of the 296 animals harbored the organism in the external nares, 267 or 90.2 per cent in the upper trachea, 244 or 82.4 per cent in the lower trachea and 115 or 38.9 per cent in the lung. The nasal cavity showed the highest percentage (94.2%) in isolation of the organism among the sites examined. Positive cultures were obtained less frequently from the tonsil, pharynx and larynx than from the nose and trachea, indicating 52.5 per cent, 43.8 per cent and 75.0 per cent of the infected animals, respectively. All the liver, spleen and kidney samples were sterile. From these findings, the nasal cavity and trachea, especially upper site of it, were found to be the favored localization-sites of *B. bronchiseptica* in guinea pigs. Thus, it was proved that nearly 90 per cent of infected animals was detected by examination on the external nares.

3. Seasonal variation in the infection of *B. bronchiseptica*

Bacteriological survey was repeatedly made on

Table 2. Monthly examination of *B. bronchiseptica* in contaminated guinea pig colonies

Month	Year					Total	%
	1962	1963	1964	1967	1968		
Jan.		58/308	204/1017	11/46	64/179	337/1550	21.7
Feb.		13/302	343/1498	34/270	36/172	426/2242	19.0
March		230/947	159/1048		44/236	433/2231	19.4
April	1/150	40/173	72/581	82/785	277/646	472/2335	20.2
May	1/149	24/163	11/327	10/13	65/264	111/916	12.1
June	16/227	18/36		31/280	38/313	103/856	12.0
July	3/120	22/274		22/702	24/514	71/1610	4.4
Aug.	0/129	3/323		22/615	1/542	26/1609	1.6
Sep.	4/197	10/300		25/697	11/555	50/1749	2.9
Oct.	0/210	0/10		76/699		76/919	8.3
Nov.	8/83	64/478		116/645		188/1206	15.6
Dec.	3/10	69/601		114/684		186/1295	14.4

Numerator : Number of animals positive

Denominator : Number of animals examined

4 contaminated breeding colonies and purchased stock in our animal house from April, 1962, to May, 1964, and from January, 1967, to September, 1968, in order to examine the seasonal incidence of *B. bronchiseptica* infection. Samples were usually taken from the external nares by swabbing, but sometimes additional organs such as the nasal cavity, trachea and lung of sacrificed animals were also examined. Results are shown in Table 2.

A definite seasonal variation was observed in infection of the organism, indicating high incidence in January to April but low incidence in July to September. Isolation rate of the organism in the summer months dropped to 1-5 per cent from about 20 per cent in the winter and early spring months. Such a variation was obtained from year to year and more evident in young animals than in old ones.

Pathological findings

During the course of bacteriological examination, it was noticed that the lung was frequently affected by lesions of various degrees not only in guinea pigs infected with *B. bronchiseptica* but also in uninfected ones. In the present study, therefore, two hundred and thirty-two guinea pigs were taken at random from contaminated colonies and their lungs were submitted to both bacteriological and pathological examinations, to discriminate the lesions associated with the organism from others.

Of the 232 guinea pigs, 138 showed apparent lung lesions of various degrees, while the remaining 94 were regarded to have healthy lung, although minor abnormalities such as epithelial changes were microscopically observed in a considerable number of them. Based on gross and histopathological findings, the lung lesions

Table 3. Relationship between lung lesion and isolation of *B. bronchiseptica*

Lung lesion	No. of lung samples		Isolation rate of the organism in per cent
	showing lesion	yielding the organism	
Bronchopneumonia	45	37	82.2
Focal inflammatory lesions	93	22	23.7
No lesion	94	6	6.4

of the 138 animals could be divided into 2 different types, one of which was bronchopneumonia and the other focal inflammatory lesions. The relationship between the lung lesions and *B. bronchiseptica* infection is listed in Table 3. The organism was isolated from 37 (82.2 per cent) of 45 lung samples suffering from bronchopneumonia. On the contrary, the organism was only found in 22 (23.7 per cent) of 93 lungs with focal inflammatory lesions and in 6 (6.4 per cent) of 94 lungs of normal appearance. No other pathogenic bacteria were demonstrated in all of the lung samples. These findings indicated that the lung lesion resulted from *B. bronchiseptica* infection was bronchopneumonia and the focal inflammatory lesions seemed to be independent of the infection. Gross and histopathological figures of these lesions were as follows.

Bronchopneumonia : Bronchopneumonia generally developed in the surrounding tissue of bronchus on hilum of the lung and often extended to a whole lobe. The lesion appeared commonly in the intermediate lobe and also less frequently in upper and middle lobes of either lungs, but lower lobes were rarely affected. The affected areas were slightly enlarged, airless and of fairly firm consistence (Photo 1). Surface of sectioned tissue was smooth or slightly granular. Color of the pneumonic areas varied from dark-red or brown to grayish yellow. Microscopically, the lesion contained variable nature of the alveolar exudate, consisting of segmentonuclear neutrophilic leucocytes and a small amount of desquamative epithelial cells. The pneumonic foci were distinctly localized around bronchioles. Small bronchi located in the pneumonic foci were generally inflamed, showing moderate infiltration of the bronchial mucosa and accumulation in their lumens of an exudate composed of leucocytes and mucus. Moderate epithelial desquamation occurred sporadically (Photos 2 and 3). The lumens of large bronchi usually contained a small amount of exudate.

Focal inflammatory lesions : These lesions were apparently atelectasis. They were mainly localized on the hilum of the lung, but occasionally seen along the bronchial tubes in different

parts of the lung (Photo 4). Color of the lesions was reddish brown. Surface of sectioned tissue was smooth and no presence of exudate from the affected areas was seen. Histopathological examination of the lesions revealed peribronchial and alveolar inflammation. The lesions were found in the connective tissue around the large bronchi, blood vessels and alveolar tissue. In cellular stage, edema and cellular exudate of plasma cells, macrophages and sometimes of giant cells were seen particularly in the interalveolar septa (Photo 5). In the chronic stage, extensive fibrosis appeared in the peribronchial and perivascular tissues, but the alveolar tissue was rarely affected. Many of the affected bronchi were almost completely obstructed either by subepithelial fibrosis or destruction of the whole bronchial wall (Photo 6).

Serological findings

1. Antigen for agglutination test

Several antigens for tube agglutination test were prepared from strain 154N and examined on their usefulness in serological diagnosis of guinea pigs infected with *B. bronchiseptica*, using sera taken from 5 infected and 3 uninfected animals. The antigens were live cultures grown in or on trypticase soy broth (TSB), nutrient broth (NB), peptone water (PW) and trypticase soy agar (TSA), formalin killed TSB and TSA cultures, TSB cultures heated at 60°C 100°C and 120°C, and TSA culture treated with alcohol. All the antigens were prepared from 20 hour cultures at 37°C.

Among the antigens, the live TSB culture reacted most sensitively with the sera of infected animals, and no agglutination was observed in the sera of uninfected animals as well as in antigen control of trypticase soy broth suspension. Live TSA culture was comparable to the TSB culture in the agglutinability but occasionally showed spontaneous agglutination in antigen control. Live NB culture and TSB culture heated at 100°C were not able to use as antigen owing to their spontaneous agglutinabilities. Live PW culture also seemed to be useless, because it agglutinated in low dilutions of the sera taken from uninfected animals, though antigen control

was negative. Spontaneous agglutination was not observed in all the other antigens, formalin killed, heated at 60°C and 120°C or treated with alcohol, but their reactivities to the sera of infected animals were not so strong as the live TSB culture. Especially, a remarkable reduction of agglutinability occurred in antigens heated at 120°C or treated with alcohol.

On the other hand, an effect of incubation time on agglutinability was examined on live TSB cultures which were incubated for 20, 48 and 72 hours at 37°C and then adjusted the concentration to McFarland's nephelometer no. 3. These cultures were mixed with sera of 5 infected guinea pigs, which were used in the preceding experiment, and submitted to the agglutination test. It was found from this experiment that as incubation time was prolonged, the agglutinabilities of the cultures were gradually reduced. In all sera, agglutination titers obtained by 20 hour culture were usually 2 to 4 times higher than those obtained by 48 and 72 hour cultures, respectively.

Based on all the results presented here, a decision was taken to use live 20 hour TSB culture as an antigen in subsequent works.

Some experiments were performed to learn whether various strains of *B. bronchiseptica* of guinea pig origin could be used as antigens. Six strains including the strain 154N and the five infected guinea pig sera used in the foregoing

experiments were employed in this study. Two of the strains were isolated from lung lesions, one from the trachea and three from the nose of naturally infected animals, and they were maintained on nutrient agar for various periods ranging one to 13 months. Results indicated that there was no difference in reactivities of the 6 strains, showing that they agglutinated in same dilutions of the sera of infected animals. None of the strains, of course, agglutinated spontaneously in sera of uninfected animals as well as in antigen controls. On the other hand, the absorption test was carried out on the sera of infected animals, using the 6 strains. It was found that all agglutinins reacted with strain 154N were removed by each of the strains without repeated absorption. Results of the 2 experiments seem enough to be expected that every strain of *B. bronchiseptica* can be used as an antigen in serological diagnosis, if it is originated from guinea pigs.

2. Practical use of the agglutination test

Attempts were made to use the agglutination test in diagnosis of *B. bronchiseptica* infection of guinea pigs. An antigen was a live 20 hour TSB culture of strain 154N. Sera were taken from hundreds animals which were collected at random from contaminated and uncontaminated colonies. Parallel examinations were also made bacteriologically and pathologically on respiratory tracts of the animals, according to the

Table 4. Agglutination titer of guinea pigs infected and uninfected with *B. bronchiseptica*

Source of animals	Isolation of organism	Broncho-pneumonia	No. of animals examined	Agglutination titer										Per cent * of positive animals
				<10	10	20	40	80	160	320	640	1280	2560	
Contaminated colonies	Positive in both trachea and lung	{ Positive	98	10	11	19	15	19	10	3	5	4	1	78.6
		{ Negative	18	2	1	4	1	2	4	1	2	1	83.3	
	Positive in trachea but negative in lung	{ Positive	18	2	1	4	3	1	2	1	3	1	83.3	
		{ Negative	100	20	25	16	19	8	4	3	2	3	55.0	
	Negative in both trachea and lung	{ Positive	6	4					1	1			33.3	
		{ Negative	96	49	21	13	6	2	4	1			27.1	
Uncontaminated control colonies			461	430	31									0

* Titers of 1 : 20 and higher were regarded as positive.

procedures described in this paper. Usefulness of the agglutination test was evaluated in comparison with the two other examinations in Table 4. In 116 animals yielded the organism in both the trachea and lung, around 80 per cent of them showed agglutination titers higher than 1:10, ranging from 1:20 to 1:2,560, regardless the development of bronchopneumonia. Agglutination titers of animals bearing the organism in the trachea but not in the lung were 1:20 or higher in 83.3 per cent of animals with bronchopneumonia and in 55.0 per cent of those without the lesion. Thus, 70 or 59.3 per cent of 118 animals showed titers ranging from 1:20 to 1:1,280. Many of animals negative in bacteriological examination showed low titers. On the other hand, agglutination titers of 461 guinea pigs obtained from uncontaminated colonies were 1:10 or lower without exception. It is also worthy to note that a gradual rise in titer was observed from young to adult in this uncontaminated group but never exceeded 1:10. From these findings, agglutination titers below 1:20 were considered to be within normal range and 1:20 or higher was regarded as significant to diagnose the infection.

3. Periodical observation on the antibody production

Following experiments were performed to examine the production and persistence of the anti-

bodies after the initiation of *B. bronchiseptica* infection in experimentally infected guinea pigs. Two million cells of 18 hour blood agar culture were smeared by a loop in the external nares of every 50 guinea pigs weighing around 300g which were obtained from some uncontaminated colonies. The inoculated animals were sacrificed five by five on given days for bacteriological, pathological and serological examinations. Results of the agglutination test are shown in Fig. 1, comparing with those of the other two examinations. The organism invaded and grew abundantly in the trachea till the 6th day after inoculation and in the lung between the 6th and 15th days producing the bronchopneumonia. A rise of the agglutination titer was not observed till the 13th day when four of 5 animals sacrificed showed positive titer of 1:20. The titers of all animals turned positive on the 15th day, distributing from 1:40 to 1:160. These figures lasted at least to the 70th day, though the organism disappeared gradually from the lung and the bronchopneumonia was renewed by normal tissue in some animals already on and after the 30th day.

DISCUSSION

Bacteriological examination of the lung has been generally used for diagnosis of *B. bronchi-*

Fig. 1 Periodical observation of agglutinin production in guinea pigs inoculated intranasally with 10^6 cells of *B. bronchiseptica*

*Number of animals positive/Number of animals examined

septica infection in guinea pigs. The present study, however, revealed that the organism usually located in either or both the nose and trachea and only less than 40 per cent of all infected ones including carriers were detectable by bacteriological examination on the lung. Ganaway et al. [8] also obtained the similar results in an epizootic of *B. bronchiseptica* infection in the Strain 13 guinea pig and claimed that carrier state could be detected accurately only at post-mortem and the trachea was the organ of choice for such a determination.

On the other hand, few attempt of bacteriological diagnosis has been made on living guinea pigs. The external nares are only places to be used for this purpose. Ganaway et al. [8] tried to isolate the organism from several parts of the respiratory tract of seven infected guinea pigs, using 5% sheep blood agar, and failed to demonstrate the organism in the external nares of all the animals except one. Quite different results, however, were obtained in the present study, in which swab samples of the external nares taken from many infected guinea pigs were cultured on MacConkey agar. The organism could be detected by this method in more than 80 per cent of the infected animals and thus, the external nares were considered to be available for diagnosis of infected animals under living conditions. A part of disagreement between their and our results probably due to difference of culture media used. In the examination of nasal samples, MacConkey agar appeared to show higher yield of *B. bronchiseptica* than blood agar as shown in the present paper. Woode and McLeod [18] also used successfully MacConkey agar, which was modified by addition of glucose and some antibiotics, as a selective medium for isolation of *B. bronchiseptica* from the nose of guinea pigs.

Horie et al. [10] and Griffin [9] reported a seasonal variation in the occurrence of *B. bronchiseptica* infection in guinea pigs and rabbits, respectively, indicating a low incidence during the summer months. The similar variation was also found in the present study. Monthly examination revealed the fact that

many infected animals were detected during October or November to June of the next year with the highest population in January to April, whilst occurrence of the infection decreased in July, August and September. It is generally accepted that the appearance of *B. bronchiseptica* infection is provoked by some form of stress such as malnutrition and poor ventilation, and Smith [16] stated that the cold temperature of winter might be responsible for the incidence of pneumonic lesions. In summer and winter, no remarkable difference was noted in husbandry of our guinea pig colonies, such as feeding, caging and breeding except weather condition. To explain the reason of this variation, further studies will be needed.

An accurate diagnosis of *B. bronchiseptica* infection is not always expected by the demonstration of the lung lesion, partly because of lack of characteristic figures distinguishable from other infectious diseases due to *Pneumococcus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae* or *Pasteurella* Spp. [3] and partly because of the fact that the isolation of the organism from the lung was positive in only less than 40 per cent of infected animals. Moreover, it should be noticed that two different types of lesions, bronchopneumonia and focal inflammatory lesions, were commonly found in the lung of guinea pigs. Bronchopneumonia was closely associated with *B. bronchiseptica* infection but the focal inflammatory lesions seemed to be independent of the infection. Although etiology of the latter could not be determined in the present study, Franks and Chesterman [6] suggested that they might be caused by a number of different stimuli such as dust and vegetable matter, presumably derived from feed or bedding.

As far as we know, no attempt has been made on serological diagnosis of *B. bronchiseptica* infection in guinea pigs. The present study indicated that the tube agglutination test seemed to be useful to a certain extent for detection of infected animals, especially to know the severity and the stage of the infection. Rise of agglutination titer occurred 13–15 days after initiation of the infection and usually accompanied with

the invasion of the organism into the lung, developing bronchopneumonia. Although positive titers, 1:20 and higher, were also obtained in a half of animals bearing the organism in the respiratory tract except the lung and in about 30 per cent of those harboring no organism, they were considered to be on the stage of recovery from the infection.

In the agglutination test for diagnostic use, choice of antigen is of most importance. The antigen is required to have specific and strong reactivity with serum antibodies of infected animals as well as no spontaneous agglutinability. Among 10 antigen preparations compared in the present study, live bacteria grown in trypticase soy broth for 20 hours was the best in satisfying the above requirements. The comparative experiments also stressed the importance of culture medium and incubation period for bacterial growth in antigen preparation, but no difference of agglutinability was observed among several strains isolated from guinea pigs. On the other hand, it should be noticed that the agglutinability of antigen was reduced by heating. In antigenic analysis of *B. bronchiseptica*, Nakase [14] proved the presence of capsular thermolabile L antigen, flagella thermolabile H antigen, surface slightly thermostable (stable at 100°C for one hour) S antigen and somatic thermostable O antigen. We performed the absorption test on 5 sera of infected guinea pigs using a culture heated at 100°C and discovered that although slight decrease in agglutination titer was observed in the absorbed sera, major parts of antibodies remained unabsorbable and agglutinated the live culture (unpublished data). These results suggested that antibodies available for serological diagnosis might be produced by either or both of the thermolabile capsular L and flagella H antigens of the organism.

SUMMARY

Bacteriological, pathological and serological examinations were made on guinea pigs infected with *B. bronchiseptica* to know characteristic features of the infection and to obtain an accu-

rate diagnosis. 1. In bacteriological examination, it was recommended that nasal samples should be cultured on MacConkey agar but the trachea and lung on blood agar. 2. The nasal cavity and upper trachea were favorite sites of localization of the organism, and the presence of the organism in the lung was proved in only 40 per cent of infected animals. The external nares were the places suitable for bacteriological diagnosis of living animals, indicating that more than 80 per cent of infected animals could be detected by the examination of the places. 3. Seasonal variation was observed in prevalence of the infection, indicating a high incidence from winter to spring and a low incidence in summer. 4. Two types of lung lesion, bronchopneumonia and focal inflammatory lesions, were frequently observed in guinea pigs autopsied in the study. The bronchopneumonia was closely associated with *B. bronchiseptica* infection, but the other seemed to be independent of the infection. 5. Diagnostic use of the tube agglutination test was successfully made to a certain extent by using a live 20 hour trypticase soy broth culture as an antigen and regarding titers 1:20 and higher to be positive. Positive titers were obtained in about 60 to 80 per cent of animals bearing the organism in the trachea or in both the trachea and lung. Antibody production became detectable 13-15 days after initiation of the infection and positive titers lasted longer than two months.

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Explanations of Photographs

- Photo 1. Bronchopneumonia. Ventral view of the lung with multilobular lesions.
- Photo 2. Bronchopneumonia. The small bronchiole and alveoli.
Exudate is mainly consisted of neutrophilic leucocytes.
- Photo 3. Bronchopneumonia. High-power view of the alveoli.
Exudate contained neutrophilic leucocytes and desquamated epithelial cells.
- Photo 4. Focal inflammatory lesions. Atelectasis-like lesion of the right intermediate lobe (arrow).
- Photo 5. Focal inflammatory lesions. Peribronchial and periarterial infiltration of small round cells.
- Photo 6. Focal inflammatory lesions. Peribronchial and subepithelial fibrosis of the bronchus.
The lumen is markedly contracted.

モルモットの気管支敗血症菌感染の診断に関する

2, 3 の知見について

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モルモットの気管支敗血症菌感染の特徴を知り、適正な診断法を得るために、細菌、病理及び血清学的な立場から検討を加えた。その成績を要約すると次の通りである。1)細菌学的な検査を行なうに当って、鼻腔材料はマツコンキー培地に、気管及び肺の材料は血液寒天培地に培養することを推奨した。2)本菌の好占部位は鼻道及び上部気管であって、肺からの菌の検出は感染動物のわずか40%において陽性であった。また、鼻前庭を検査することによって感染動物の80%以上が摘発されることから、本症の生前診断にこの部位が利用出来ることを知った。3)本症の発生には一定の季節的変動が認められ、冬から春にかけては高い発生率を示したが、夏季には減少した。4)今回剖検したモルモットの肺には二つの異なる

病変、すなわち、気管支肺炎と **focal inflammatory lesions** がしばしば観察された。この中気管支肺炎の形成は気管支敗血症菌の感染と密接な関係を有したが、**focal inflammatory lesions** は本菌の感染とは無関係に形成された。5)**trypticase soy broth** に20時間培養した生菌を抗原とし、20倍以上の凝集価を陽性となすならば、試験管凝集反応は本症の診断にかなり有用であることがわかった。すなわち、この方法によって気管或は肺に本菌を保有するモルモットの60～80%が陽性凝集価を示した。また、凝集抗体は感染が始まってから13～15日で検出可能となり、陽性反応は少なくとも2カ月以上にわたって持続した。