EXPERIMENTAL RESPIRATORY INFECTION WITH 'PASTEURELLA MULTOCIDA' AND BORDETELLA BRONCHISEPTICA' IN RABBITS

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Eight- to ten-week-old offspring of a colony of specific pathogen-free rabbits were exposed to cultures of Pasteurella multocida and Bordetella bronchiseptica. Two groups of nine animals were exposed to either bacterium intranasally and sacrificed 2, 7, 14, and 21 days postinoculation. Five of nine rabbits in each group developed a mucopurulent nasal discharge 4 to 7 days postinoculation. The remaining four rabbits in each group failed to develop clinical signs. The gross and microscopic lesions did not differ significantly in character or distribution among the inoculated rabbits. The

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20. Abstract

infection was characterized by an acute upper respiratory syndrome accompanied by a mild bronchopneumonia.

PREFACE

The work described in this report was authorized under Project 1W062116AD21, Medical Fffects of Chemical Agents. This work was started in February 1974 and completed in March 1974.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council.

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CONTENTS

EXPERIMENTAL RESPIRATORY INFECTION WITH PASTEURELLA MULTOCIDA AND BORDETELLA BRONCHISEPTICA IN RABBITS

1. INTRODUCTION

1.1 Respiratory disease occurs quite commonly in laboratory rabbits and, although the etiologic agents involved are multiple, the organisms isolated most frequently are Pasteurella multocida and Bordetella bronchiseptica.[1-3] The respiratory disease complex in rabbits may vary from a mild, chronic, mucopurulent upper respiratory infection (snuffles) to a more acute to subacute bronchopneumonia (enzootic pneumonia) leading to high mortality.[1] The incidence of pneumonia may vary from 20% to 50% in conventional colonies.[2-4] Since the disease complex is enzootic in many laboratory colonies, experimental studies describing the lesions produced by P. multocida and B. bronchiseptica are limited. A recent report described the lesions produced by P. multocida after the intratracheal inoculation of young rabbits.[5]

1.2 The purpose of this report is to describe the clinical and necropsy findings in rabbits experimentally exposed to P. multocida and B. bronchiseptica intranasally.

2. MATERIALS AND METHODS

2.1 Inoculation of Animals. Rabbits used in this study were 8- to 10-wk-old offspring of a colony of rabbits that had been caesarean-derived and barrier-reared at Edgewood Arsenal since 1968 [Ea: (NZWxFG) BR]. The parent colony has been found to be free of contamination with P. multocida, B. bronchiseptica, and Mycoplasma spp. through periodic microbiologic and necropsy monitoring procedures. The experimental group assigned, inoculum used, and necropsy schedule are outlined in table 1. Rabbits were transferred to conventional housing areas and inoculated intranasally, using a sterile serologic pipette and rubber bulb, with 5.0×10^9 P. multocida and 3.5×10^{10} B. bronchiseptica organisms suspended in 0.5 ml of sterile saline. Sham inoculated controls received 0.5 ml of sterile saline by the same route. To simulate a mild stressful condition, all rabbits received intramuscular injections of 25 mg/kg of hydrocortisone succinate* for 3 successive days beginning 1 day before inoculation.

TABLE 1. EXPERIMENTAL PROTOCOL

Group	Number of rabbits	Inoculum	No. of rabbits necropsied			
			2 days	7 days	14 days	21 days
1	9	P. multocida	2	2	2	3
2	9	B. bronchiseptica	2	2	2	3
3	4	Saline control	1	1		2
4	3	Uninoculated		1		2

*Solu-Cortef® 100 mg Mix-O-Vial - UpJohn Company, Kalamazoo, Michigan.

2.2 Preparation of Inoculum and Reisolation of Pasteurella and Bordetella. B. bronchiseptica was isolated from the pneumonic lungs of a conventional rabbit, characterized, and propagated as a laboratory stock culture. P. multocida was isolated from the nasal cavity of a rabbit with clinical signs of "snuffles." Prior to inoculation, both organisms were grown in brain-heart infusion broth shaker cultures for 24 hr at 37°C. Pellets were formed by centrifugation of broth suspensions at 2000 rpm for 15 min and resuspended in sterile saline for inoculation of rabbits. The concentration of live organisms was established using tenfold serial dilutions of a sample of inoculum grown on sheep blood agar plates for 24 hr at 37°C. Colonies were counted using a standard colony counter. A second sample was withdrawn to test the purity of the suspension. Bacteriologic identification of all samples was made using the differential biochemical tests described by Cowan and Steel.[6] Tissue specimens collected during necropsy of experimental rabbits were homogenized in sterile broth. They were then inoculated onto a trypticase soy agar plate with 5% sheep blood and a MacConkey agar plate and into a tube of brain-heart infusion broth. After 24 hr incubation at 37°C, suspect colonies were identified biochemically as previously referred to in the text.

2.3 Necropsy Procedures. All rabbits were killed by intraperitoneal injection of a concentrated barbiturate solution.* Tissue samples were collected from the nasal cavity, trachea, and lungs for histologic and bacteriologic examination. Prior to opening the thoracic cavity, the trachea was ligated at two points to prevent collapse of lungs. Tissues collected for bacteriologic examination were processed as previously described. Specimens for histologic examination were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 6μ, and stained with hematoxylin-eosin (H&E).

3. RESULTS

3.1 Clinical Signs. Five rabbits from each inoculated group (5/9) developed a mild to moderate mucopurulent nasal discharge 4 to 7 days postinoculation which persisted until the animals were sacrificed. No clinical signs were noted in the remaining four rabbits in each group (4/9) nor in the control rabbits.

3.2 Necropsy Findings.

3.2.1 Gross and microscopic lesions observed in both inoculated groups were similar in character and distribution. The degree of lung involvement in the B. bronchiseptica group was greater than in the P. multocida group in most cases. Severe lesions in the lungs were not observed within either group. No significant gross or microscopic lesions were seen in either control group.

3.2.2 Gross lesions were observed in rabbits killed 7, 14, and 21 days postinoculation. In the rabbits killed on the seventh day, the nasal turbinates were congested, swollen, and contained a mucopurulent exudate. The trachea was congested. The lungs were also congested and contained focal, scattered, reddish brown areas of consolidation. Consolidated areas were found mainly in the ventral portion of the cardiac lobes with minimal involvement of the cranioventral portion of the diaphragmatic lobes. The lesions at 14 and 21 days did not differ from those observed at 7 days except a greater quantity of nasal exudate was present and the degree of lung involvement

*Lethal solution - Elanco, Indianapolis, Indiana.

was more extensive in B. bronchiseptica inoculated rabbits than in P. multocida inoculated rabbits. A few focal, depressed areas were in the lungs of some rabbits 21 days postinoculation. A tabulation of clinical signs, gross and microscopic lesions, and bacterial isolation is shown in table 2.

TABLE 2. INCIDENCE OF CLINICAL SIGNS, LESIONS, AND POSITIVE REISOLATION OF BACTERIA

Inoculum	Clinical signs*	Lesions		Reisolations of bacteria		
		Gross	Microscopic	Nasal cavity	Trachea	Lung
	No. of positive/No. of inoculated					
P. multocida	5/9	5/9	5/9	4/9	0/9	1/9
B. bronchiseptica	5/9	6/9	6/9	4/9	6/9	3/9

*Mucopurulent nasal discharge.

3.2.3 No significant microscopic lesions were seen in sections from rabbits killed 2 days postinoculation. The number of animals with microscopic lesions at each interval is shown in table 3. Seven days postinoculation, lesions observed in rabbits in both inoculated groups consisted of a mild infiltration of heterophils into the nasal mucosa and submucosa, aggregates of heterophils and debris in the lumina of the nasal sinuses, and congestion of the tracheal mucosa. Scattered foci of mixed inflammatory cells infiltrated the peribronchial tissue and in some cases invaded the bronchial epithelium. There was also minimal septal cell thickening with increased cellularity in the interstitium.

TABLE 3. NUMBER OF ANIMALS WITH MICROSCOPIC LESIONS AT EACH INTERVAL

Inoculum	Incidence of lesions				
	2 days	7 days	14 days	21 days	Total
	No. of positive/No. of necropsied				
B. bronchiseptica	0/2	2/2	2/2	2/3	6/9
P. multocida	0/2	1/2	2/2	2/3	5/9

3.2.4 A greater involvement of the nasal mucosa and lung was evident at 14 days postinoculation. There was an increase in fibrinous exudate and inflammatory cells in the nasal sinuses (figure 1), and areas of the nasal mucosa were ulcerated (figure 2). In affected lung parenchyma, a marked proliferation and disruption of peribronchial lymphoid tissue was noted (figure 3). In addition to a pronounced thickening of septal cells, there was a mononuclear inflammatory exudate containing some erythrocytes and a moderate number of macrophages in the alveolar spaces (figure 4). Although the distribution of lung lesions remained patchy, these changes were more pronounced in the B. bronchiseptica inoculated rabbits than in those given P. multocida.

3.2.5 Lesions observed 21 days postinoculation were similar to those observed at 14 days except the peribronchial cellular proliferation was more severe. Pronounced perivascular cellular infiltration (figure 5) and focal areas of fibroblastic and histocytic proliferation were observed in the lung. No significant lesions were seen in tracheal sections taken at 14 and 21 days.

3.2.6 Reisolation of the organisms from organ samples are summarized in table 2. These figures represent organ isolations in animals with lesions except that B. bronchiseptica was isolated from the trachea of two inoculated rabbits that had no gross lesions or microscopic lesions. Cultures of tissue samples from control animals were consistently negative for B. bronchiseptica and P. multocida throughout the study.

4. DISCUSSION

4.1 Pasteurella spp and Bordetella spp have been incriminated as the primary etiological agents in a variety of diseases in small laboratory animals.

4.2 The disease in rodents includes respiratory and uterine infections in mice[3-7] and pneumonia in rats and guinea pigs.[3,8,9] In rabbits, P. multocida may produce a chronic rhinitis, otitis media, conjunctivitis, subcutaneous and uterine abcesses, and bronchopneumonia.[1,2,10] B. bronchiseptica has been associated primarily with chronic rhinitis and bronchopneumonia.[3,4] Both organisms are known to occur in the respiratory tract of clinically healthy rabbits, and stressful conditions are considered necessary for overt disease to appear.

4.3 In this study, the mucopurulent nasal discharge observed in rabbits inoculated intranasally with each organism was consistent with the predominant clinical signs seen in "snuffles." The fibrinopurulent rhinitis with ulceration of the mucosa observed microscopically did not differ significantly between the two groups. Similar lesions are also found in the naturally occurring disease. Flatt and Dungworth[5] were able to produce a severe fibrinopurulent bronchopneumonia with some deaths in young rabbits by intratracheal inoculation of 10^7 P. multocida organism. Although there were no deaths in the present study, the microscopic changes in the lung were similar in character though not as extensive as those described by Flatt.

4.4 The early involvement of the bronchial and peribronchial tissues at 7 days postinoculation and the later involvement of the lung alveolar tissue at 14 and 21 days indicate an extension of an infection originating primarily in the bronchial tree. The very striking

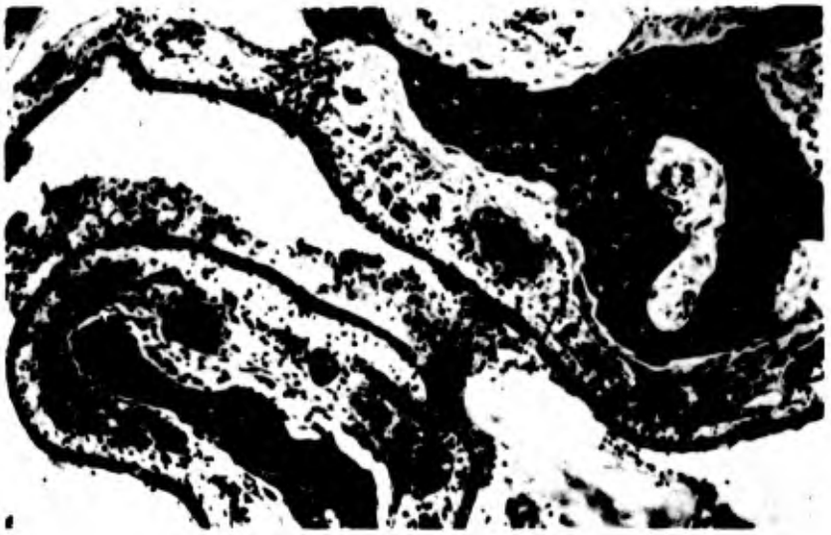

Figure 1. Fibrinopurulent Rhinitis in Nasal Cavity of Rabbit 14 Days after Inoculation with P. multocida. Note exudate in lumen and congestion and edema of adjacent mucosa and submucosa. H & E (original magnification) 27X.

Figure 2. Fibrinopurulent Rhinitis with Erosion of Nasal Mucosa in Nasal Cavity 14 Days after Inoculation with B. bronchiseptica. H & E (original magnification) 70X.

Figure 3. Lung of Rabbit 14 Days after Inoculation with B. bronchiseptica. Note peribronchial lymphoreticular aggregate disrupting bronchiolar epithelium. H & E (original magnification) 27X.

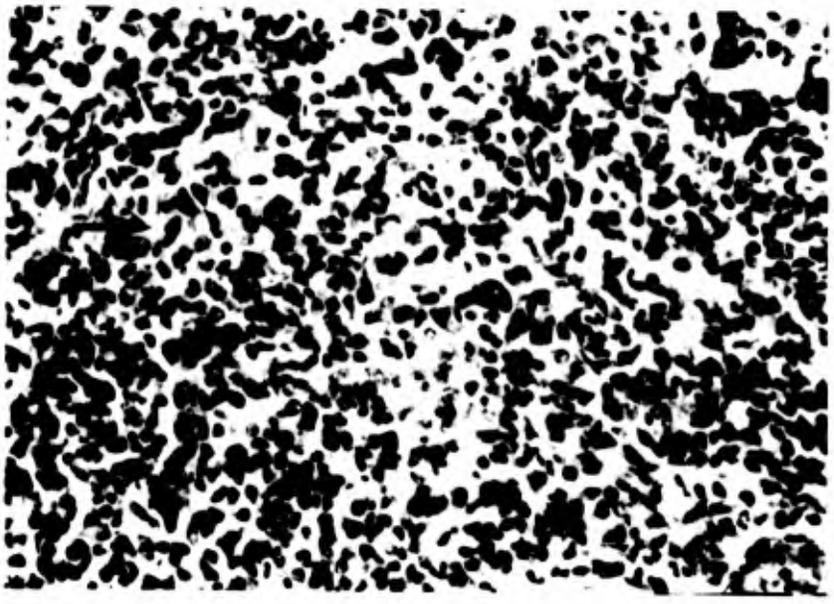

Figure 4. Lung from Rabbit in Figure 3. Alveolar septal thickening (arrow) and predominantly mononuclear cell infiltration in alveoli spaces. H & E (original magnification) 70X.

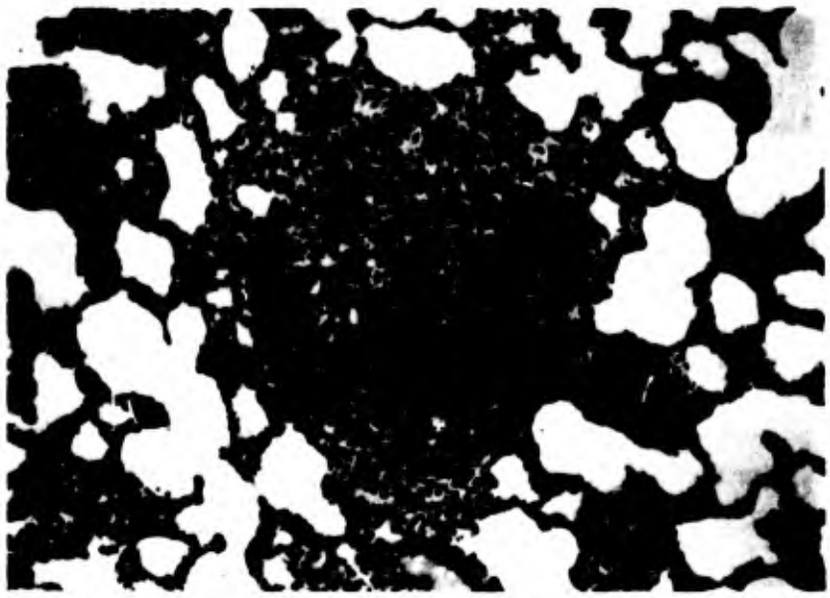

Figure 5. Lung from Rabbit 21 Days after Inoculation with B. bronchiseptica. Lymphoreticular cell aggregation and perivascular cuffing. Hypercellularity of adjacent alveolar septa. H & E (original magnification) 27X.

lymphoreticular perivascular reaction has been previously described[5] and it is consistent with lesions observed in the spontaneous disease.[2]

4.5 Isolation of B. bronchiseptica from the trachea of six of nine (66%) inoculated animals suggests a possible site of multiplication without significant damage to the organ. P. multocida was isolated from the nasal cavities of four of nine (44%) rabbits. The recovery rate from lung samples was low in both groups. This may have been due to the patchy distribution of lesions or the amount of tissue submitted for examination.

4.6 The results of this study indicate that respiratory disease in rabbits may be caused by either B. bronchiseptica or P. multocida under stressful conditions. It does not appear possible to differentiate infection produced by either organism based upon clinical signs, gross lesions, or histologic findings since the location, distribution, and characteristics of the lesions are quite similar.

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