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PORCINE CONTAGIOUS PLEUROPNEUMONIA

II. Studies of the Pathogenicity of the Etiological Agent, Hemophilus pleuropneumoniae

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In the preceding paper (1), *Hemophilus pleuropneumoniae* was shown to be the causative agent of a frequently fatal porcine contagious pleuropneumonia (PCP) occurring in Argentine swine. Further study of the pathogenicity of this organism has shown it to have some unexpected and interesting properties as concerns its behavior when administered to animals subcutaneously instead of intranasally. It is the purpose of the present paper to outline the rather striking differences that this organism shows in its pathogenicity for swine, contingent on the route by which it is administered, and further to indicate the exquisite sensitivity of swine to the organism when given by way of the respiratory tract.

Materials and Methods

Swine.—Grade Berkshire or Landrace swine, 7 to 19 weeks of age, were used in this work.

Culture Media.—The *H. pleuropneumoniae* employed in these experiments was grown either in Levinthal medium or proteose-peptone broth fortified with glucose and DPN, as described by White and Smith (2), or in defibrinated horse blood at the base of a plain agar slant, as used earlier for the cultivation of *Hemophilus influenzae suis* (3). Counts of viable organisms were made by plating suitably diluted cultures in pour plates of the White and Smith medium containing 1.5 per cent agar or Levinthal agar. In the fluid media we have employed in the present study, *H. pleuropneumoniae* reaches its peak titer of about 1×10^9 viable organisms per ml within about 12 hours of incubation.

Pathogenicity of H. pleuropneumoniae for Swine when Administered Intranasally.—

In the preceding (1) paper, it was shown that swine were very susceptible to *H. pleuropneumoniae*, either in pure culture or in suspensions of infected lung, if it was administered intranasally. However, no effort to quantitate the organism's pathogenicity was made nor was any consideration given to the possibility that the two colony types formed by *H. pleuropneumoniae* (1) (dense "waxy" or smooth "iridescent" on Levinthal agar) might yield organisms

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of differing virulence. This has now been done and the findings are outlined in Table I.

Doses of *H. pleuropneumoniae*, isolated from swine with experimental porcine contagious pleuropneumonia (PCP), ranging from as few as 100 to as many as 34,000,000 organisms have

TABLE I

Pathogenicity of H. pleuropneumoniae for Swine Intranasally

Swine No.	No. of colony-forming *H. pleuropneumoniae* intranasally*	Results
40-31	3.4×10^7	Dead, 1st day
40-27	3.4×10^7	" , 3rd "
40-34	2.2×10^7	" , 1st "
40-33*b*	1.5×10^7	" , 2nd "
39-02	8.5×10^6	" , 1st "
38-97	8.5×10^6	" , 2nd "
40-40	5.5×10^5 (waxy)‡	" , 4th "
40-35	5.5×10^5 (iridescent)	Sick 5 days, survived
39-30	1×10^5	Dead, 4th day
40-30	1×10^5	Sick 10 days, survived
40-39	5.5×10^4 (waxy)	" 5 " , "
40-36	5.5×10^4 (iridescent)	" 9 " , killed
39-49	4.5×10^4	" 9 " , survived
40-12	4×10^4 (waxy)	" 2 " , "
40-10	4×10^4 (iridescent)	" 5 " , "
39-71	3.2×10^4	" 7 " , "
39-84	2.5×10^4	" 5 " , "
39-73	2×10^4	" 4 " , killed
39-59	1.2×10^4	" 6 " , survived
40-38	5.5×10^3 (waxy)	" 8 " , killed
40-37	5.5×10^3 (iridescent)	Negative
39-35	1.4×10^3	Dead, 3rd day
39-36	1×10^2	Sick 9 days, survived

* Administered intranasally in either 5 or 10 ml of synthetic medium of the type used for expression of competence in *Hemophilus* transformation experiments (4).

‡ "Waxy" and "iridescent" refer to the two colony types that *H. pleuropneumoniae* forms on Levinthal agar, and the suspensions designated were made up of pure cultures of one or the other colony types. The exact composition of undesignated suspensions as to colony types is unknown.

been administered intranasally to swine in 5 or 10 cc of suspending fluid. The suspending fluid employed was the synthetic medium used for expression of competence in *Hemophilus* transformation studies (4), a medium that is not deleterious to *H. pleuropneumoniae* but in which it will not multiply. Injection was by Luer syringe without needle, inserting the tip of the syringe into the nostrils and expelling about one-half of the total inoculum into each nostril while holding the animal's mouth tightly closed.

It is impossible to know exactly how many of the organisms administered intranasally in this way adhere to nasal and respiratory tract mucosa and how

many pass back into the pharynx and are swallowed. However, observation of the behavior of the swine at time of inoculation makes it evident that only a very small proportion of the organisms in the 5 or 10 ml of inoculum administered is actually retained in the respiratory tract. The number of organisms recorded in Table I as having been given intranasally, therefore, probably are several-fold higher than the number that were effectively retained in the respiratory tract. However, even with the certain loss of some organisms due to the method of administration, *H. pleuropneumoniae* proved to be highly pathogenic for swine.

Most of the swine recorded in Table I were inoculated with cultures of *H. pleuropneumoniae* that had not been recently derived from single colony isolates and hence the composition of the inoculum, as to colony types of organisms contained, is not known. However since the organisms forming the dense waxy type colonies rapidly, on serial cultivation on chocolate or Levinthal agar, yielded organisms that formed smooth iridescent colonies on Levinthal agar, it seems likely that most of the cultures used may have been predominantly or entirely of the latter type. The organisms forming the smooth iridescent colonies were stable on cultivation and bred true on serial passage on Levinthal medium. Four of the swine, as recorded in Table I, were inoculated with cultures derived from single colony isolates of the small dense waxy colony type *H. pleuropneumoniae*, while four others received cultures from single colony isolates of the smooth iridescent colony type. Both types proved pathogenic for swine and so far as could be told from the small numbers of animals employed, there was probably little or no difference in the virulence of the organisms comprising the two colony types. The failure of swine 40-37, which received 5500 of the smooth iridescent colony type organisms, to sicken while swine 40-38, receiving the same number of dense waxy colony type organisms did become ill might indicate a somewhat greater capacity of the latter type organisms to establish in the respiratory tract when administered in small numbers.

As shown in Table I, an inoculum containing 100 organisms proved infective and one containing only 1400 was lethal in one case. There was only one complete miss in the titration series, that of swine 40-37, and this has been commented on in the preceding paragraph. The regularly lethal number of *H. pleuropneumoniae* intranasally seemed to be about one-half million organisms since most of the swine receiving this number, or more, died, whereas those receiving less usually survived. Swine 39-30, which succumbed to a dose of 100,000, and swine 39-35, dying from infection with 1400 organisms, were the exceptions. So far as can be judged, therefore, from the findings presented in Table I, the infective dose of *H. pleuropneumoniae* intranasally was something less than 100 organisms, while the lethal dose was about one-half million.

In order to compare the pathogenicity of *H. pleuropneumoniae* with another swine *Hemophilus* having the same growth requirements, two swine have been inoculated intranasally with relatively enormous numbers of a culture of a

DPN-dependent strain of *H. influenzae suis* (3). Swine 40-57 was given a dose of 1.6×10^7 organisms and swine 40-54 a dose of 1.3×10^9. Neither animal showed any evidence of illness. Such findings make it clear that though *H. pleuropneumoniae* and DPN-dependent strains of *H. influenzae suis* are quite similar culturally, they are very different as regards their pathogenicity for swine.

The Failure of H. pleuropneumoniae to Induce Illness when Administered Subcutaneously.—

TABLE II

Pathogenicity of H. pleuropneumoniae Subcutaneously and Intranasally

H. pleuropneumoniae subcutaneously			Pathogenicity of same suspensions of *H. pleuropneumoniae* given intranasally		
Swine No.	Number of organisms*	Results	Swine No.	No. of organisms‡	Results
39-60	4.1×10^5	Negative	39-59	1.2×10^4	Sick 6 days, survived
39-66	1.7×10^5	"			
39-68	1×10^5	"§			
39-72	6.8×10^5	"	39-71	3.2×10^4	" 7 " , "
39-74	1×10^5	"	39-73	2×10^4	" 4 " , killed
40-24	3.4×10^8	"§	40-31	3.4×10^7	Dead 1st day

* Administered subcutaneously in the inguinal region in 1 ml of synthetic transformation medium (4).

‡ Administered intranasally in either 5 or 10 ml of synthetic transformation medium.

§ Boardy induration at site of injection.

In view of the marked pathogenicity of *H. pleuropneumoniae* intranasally, it was unexpected to find that when given subcutaneously, it had little or no obvious effect, as shown in Table II. Six swine have been injected subcutaneously with relatively massive doses of *H. pleuropneumoniae* and none of these have developed either a febrile reaction or evidence of illness. In two of them a boardy induration of the subcutaneous tissues at the site of injection developed, and the regional lymph nodes became hard and enlarged. None of the six swine had lesions in the pleurae or lungs when they were eventually killed and autopsied, indicating that the characteristic pleuropneumonia following intranasal infection had not resulted from subcutaneous inoculation with *H. pleuropneumoniae.*

As shown in Table II, four of the six bacterial suspensions given subcutaneously were also tested for intranasal infectivity in lower dosage and found by that route to be fully pathogenic for swine. It seemed apparent from these findings that *H. pleuropneumoniae* is a rather strictly pneumotropic organism and must be administered by way of the respiratory tract to induce the charac-

teristic pleuropneumonia which it causes under natural conditions. Furthermore, the results indicated that swine can withstand subcutaneously, without apparent signs of illness, a dosage of organisms that would be lethal intranasally.

Immunity Conferred by Subcutaneous Inoculation of Swine with H. pleuropneumoniae.—Viruses that show a marked tropism for one type of tissue or organ system and that are infective only by way of that particular tissue route usually induce immunity when administered by a route other than the one by which they are infective. The immunization of swine against the pneumotropic swine influenza virus by virus given subcutaneously or intramuscularly (6) or of

TABLE III

Immunity Conferred against PCP by Subcutaneous Injection of Swine with H. pleuropneumoniae

Swine No.	Immunization		Challenge		Swine No.	Control for challenge	
	No. of *H. pleuropneumoniae* subcutaneously*	Results	No. of *H. pleuropneumoniae* intranasally‡	Results		No. of *H. pleuropneumoniae* intranasally	Results
40-24	3.4×10^8	Negative	3.4×10^7	Negative	40-27	3.4×10^7	Dead, 3rd day
39-66	1.7×10^5 1×10^5	Negative "	2×10^4	Negative	39-73	2×10^4	Sick 4 days, killed
39-60	4.1×10^5	Negative	3.2×10^4	Negative	39-71	3.2×10^4	Sick 7 days, survived

* Administered subcutaneously in 1 ml of synthetic transformation medium (4).

‡ Administered intranasally in 5 or 10 ml of synthetic transformation medium.

rabbits against the dermatotropic papilloma virus by virus given intraperitoneally (7) are examples of this particular phenomenon. Since most pathogenic bacteria are not as strictly tissue-tropic as *H. pleuropneumoniae* appeared to be, it seemed of interest to learn whether this pneumotropic organism might, like the pneumotropic virus cited above (6), induce immunity when injected by a non-infective route.

Two swine that had had a single subcutaneous dose of *H. pleuropneumoniae*, and one that had had two such injections, were inoculated intranasally, together with three control swine, with *H. pleuropneumoniae.*

As shown in Table III, all three of the swine that had had an earlier subcutaneous exposure to *H. pleuropneumoniae* resisted an intranasal infection with this organism that was fully pathogenic for control animals. The protection conferred was complete, for not only did the animals fail to sicken, but at autopsy, after a period of observation, their lungs and pleurae were free of the characteristic lesions found in swine recovered from PCP (1). It seemed ap-

parent from this result that, although *H. pleuropneumoniae* failed to cause obvious illness when administered to swine subcutaneously, it did render them solidly immune to later intranasal infection with the organism. This pneumotropic bacterium thus behaved similarly to the pneumotropic swine influenza virus as regards the induction of immunity when given by a route other than the respiratory tract.

DISCUSSION

Although the minimal sensitivity of swine to infection with *H. pleuropneumoniae* cannot be known exactly, owing to the inability to determine certainly the number of organisms in a counted suspension that establish in the respiratory tract of animals to whom it is given intranasally, the experiments presented indicate that 100 or less organisms are adequate to cause PCP. The number required regularly to cause fatal infections is in the neighborhood of one-half million organisms. Some animals, for reasons that are not apparent, succumb to much smaller intranasal doses of the organism. This rather strikingly high infectivity and pathogenicity of *H. pleuropneumoniae* for swine by way of the respiratory tract contrasts with the complete lack of pathogenicity of another, and seemingly closely related swine *Hemophilus, H. influenzae suis*. *H. influenzae suis* given intranasally alone to swine is completely non-pathogenic, even when extremely large numbers are administered, and only when it is concurrently present in the respiratory tract with the swine influenza virus (5) does its pathogenicity become apparent. We thus have, in these two *Hemophili*, one that is capable, completely on its own, of eliciting marked and even fatal pathologic changes in the swine respiratory tract and another that requires the associated presence of a virus in order to manifest its pathogenicity. One can only speculate concerning the possibility that the virus may supply to *H. influenzae suis* some factor, perhaps an obscure enzyme system, that *H. pleuropneumoniae* already has incorporated as a regular part of its substance.

In addition to its very marked pathogenicity for the swine respiratory tract, there is one other feature of the behavior of *H. pleuropneumoniae* that is of interest and this concerns its relative pneumotropism. Although the organism, even in small numbers, regularly produces PCP when administered intranasally, it is innocuous, so far as concerns disease production, when given to swine subcutaneously, even in large numbers. In its failure to produce disease when given by a route other than respiratory, *H. pleuropneumoniae* behaves in a manner that we would consider generally more characteristic of a virus than of a bacterium. Furthermore, like one well known pneumotropic virus of swine, the swine influenza virus, which immunizes swine against swine influenza when given by a non-respiratory route, *H. pleuropneumoniae* immunizes swine solidly against PCP when it is administered subcutaneously.

The two features of *H. pleuropneumoniae* that have been discussed, namely its

exquisite pathogenicity for the swine respiratory tract and its relative pneumotropism, set it aside as a somewhat unique bacterium. Although with respect to its growth requirements and in its morphologic features it is certainly a bacterium of the genus *Hemophilus*, some of its other characteristics, especially its behavior as a pathogen in swine, resemble those of some viruses.

SUMMARY

Hemophilus pleuropneumoniae is highly pathogenic for swine when given intranasally. As few as 100 organisms induce characteristic porcine contagious pleuropneumonia (PCP) and, when as many as one-half million are given, the infection usually proceeds to a fatal termination. While the organism is highly pathogenic when introduced by way of the respiratory tract, it is innocuous when given subcutaneously even in large numbers. Swine that have been inoculated subcutaneously are rendered solidly immune to infection with *H. pleuropneumoniae* intranasally. The marked pathogenicity of *H. pleuropneumoniae* for swine has been contrasted with the lack of pathogenicity of another swine *Hemophilus, H. influenzae suis*. It has been pointed out that, in its high degree of pathogenicity, in its pneumotropism, and in its immunogenicity by a nonrespiratory route of inoculation, *H. pleuropneumoniae* appears to resemble certain viruses more than it does a bacterium.

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