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PORCINE CONTAGIOUS PLEUROPNEUMONIA

III. INTERRELATIONSHIP OF *HEMOPHILUS PLEUROPNEUMONIAE* TO
OTHER SPECIES OF *HEMOPHILUS*: NUTRITIONAL, METABOLIC,
TRANSFORMATION, AND ELECTRON MICROSCOPY STUDIES

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PLATES 1 AND 2

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Previous work with *Hemophilus pleuropneumoniae* has shown that this bacterium is the causative agent of a characteristic fulminating, frequently fatal porcine contagious pleuropneumonia (PCP) (1). Further studies have demonstrated that the organism is pathogenic for swine only when administered intranasally and that it is innocuous though immunogenic when inoculated subcutaneously (2). The present study compares *H. pleuropneumoniae* to certain other members of the genus *Hemophilus* in an attempt to clarify the interrelationships of this pathogen.

Materials and Methods

Bacterial Strains.—*H. pleuropneumoniae* was isolated from the lungs of swine with PCP (1). *H. parainfluenzae* (Boss), *H. influenzae* Rd. (Garf), and *H. aegyptius* (strain 15) were of human origin (3, 4).

H. influenzae suis (*H. suis*), herein referred to as *H. parasuis* since the strain (1356) selected requires diphosphopyridine nucleotide (DPN) but can form hemin (3, 5). It was isolated from the respiratory tract of a pig with swine influenza (6).

H. parainfluenzae (K-17) was a meningeal strain isolated from a steer (7).

Media.—The proteose-peptone medium is similar to that described by White and Smith (8) and contains 2 per cent proteose-peptone (Difco Laboratories, Inc., Detroit), 0.6 per cent NaCl, 0.1 per cent KNO₃, and 0.002 per cent Na₂S₂O₄. It is buffered to pH 7.6 with 0.02 M tris. When used for the growth of *Hemophilus*, it is fortified after sterilization by the addition of DPN (sterilized by Millipore filtration), sterile yeast extract, and sterile glucose (Difco products), in concentrations of 0.5 μg per ml and 0.5 and 1 per cent respectively. In the experiments reported in Table I, glucose and yeast extract were omitted from this medium. The defined medium is the simplified medium of Herbst and Snell (9) to which histidine was added (10). Sodium bicarbonate was incorporated in this medium to a concentration of 0.02 M where subsequently indicated. Levinthal medium (11) was used in the transformation studies.

Growth.—Growth was measured turbidimetrically, using a Klett photometer with a blue-green filter (band pass 500 to 600 mμ). In each case, the instrument was set to zero with un-

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inoculated medium. Tubes 13 mm in diameter were used, and viable counts were directly proportional to turbidity between 10 and 60 units (10^6 and 10^8 colony-forming units per ml).

Transformation.—Spontaneously occurring mutant populations resistant to at least 1 mg of streptomycin per ml were selected from both the smooth “iridescent” and the dense “waxy” colony types (1, 2) of *H. pleuropneumoniae*. DNA-containing fractions were prepared from the mutant populations as described by Alexander and Leidy (12). The technical procedures for the transformation experiments were those previously described by Leidy *et al.* (3, 10).

Electron Microscopy.—Heavily seeded cultures of *H. pleuropneumoniae* were grown for 18 hours in proteose-peptone medium and aliquots containing about 10^9 bacteria per ml were harvested by centrifugation. The bacteria were fixed overnight at 0°C according to the method of Ryter and Kellenberger (13) and embedded in epon 812 (14). Thin sections were cut on a Porter-Blum microtome using a diamond knife, collected on naked grids, and examined in a Siemens-Elmiskop I after staining with 0.5 per cent aqueous uranyl acetate for 30 minutes followed by alkaline lead (15). The bacterial population in these preparations was probably in the stationary phase of growth.

Difference Spectra.—Early stationary-phase cells were used to compare the oxidized and reduced states of the electron transport system. The methods of preparing bacterial suspensions and measuring the spectra have been described by White (16). The nomenclature for cytochromes follows that used by White and Smith (8).

EXPERIMENTAL RESULTS

Hemin and DPN Requirements.—

Members of the *Hemophilus* group characteristically require hemin (X factor) and/or DPN (V factor) for growth. Growth is directly proportional to hemin concentration in the range of 0.002 to 0.05 mcg per ml for the hemin-requiring species (17), and to DPN concentration in the range 0.01 to 0.1 mcg per ml for DPN-requiring *H. parainfluenzae*. The proteose-peptone medium used in this study contains less than 10^{-9} M hemin (5); any DPN present is destroyed by autoclaving. A chemically defined medium (Materials and Methods) has also been used to test for these growth requirements but is of limited use in that hemin-requiring species do not grow in the medium; a test for contaminating traces of hemin is therefore not feasible.

The data of Table I establish that *H. pleuropneumoniae*, like *H. parainfluenzae* and *H. parasuis*, requires DPN but not hemin for growth. The data also show that when small inocula (10 to 100 cells) are used, *H. pleuropneumoniae* and *H. parainfluenzae* (K-17) fail to grow in the defined medium unless bicarbonate ions (0.02 M) are present; Boss, the *H. parainfluenzae* strain of human origin, does not require added HCO_3^- . The *H. parasuis* strain did not grow in the defined medium.

Carbohydrate Utilization.—

Fermentation reactions are difficult to use as a taxonomic criterion with *Hemophilus* species since these bacteria grow in such complex media that the effects of added carbohydrate give variable results. However, for those species which grow in the defined medium, growth is dependent upon the carbohydrate added.

As shown in Table II, both *H. pleuropneumoniae* and *H. parainfluenzae* (Boss) can be differentiated from *H. parainfluenzae* (K-17) by their ability to grow in the presence of lactate, sucrose, and mannitol. The pH changes from 7.6 to 6.4 when the turbidity exceeds 50 units.

TABLE I
Comparison of Hemin and DPN Requirements of *H. pleuropneumoniae* with Other
Hemophilus Species

Medium	Turbidity*				
	<i>H. pleuro- pneumoniae</i>	<i>H. parain- fluenzae</i> (Boss)	<i>H. parain- fluenzae</i> (K-17)	<i>H. parasuis</i> (1356)	<i>H. influ- ensae</i>
Proteose-peptone†.....	0	0	0	0	0
Proteose-peptone + DPN.....	46	50	60	40	0
Proteose-peptone + hemin.....	0	0	0	0	0
Proteose-peptone + DPN + hemin..	42	51	59	41	56
Defined medium.....	0	0	0	0	0
Defined medium + DPN.....	0	150	0	0	0
Defined medium + DPN + HCO ₃ ⁻	130	170	156	0	0

* Measured after 48 hours at 37°C in 18-mm test tubes containing 5 ml medium grown from an inoculum of 10 to 100 late stationary phase cells.

† Glucose and yeast extract omitted.

TABLE II
Comparison of Carbohydrate Utilization by *H. pleuropneumoniae* and *H. parainfluenzae*

Carbohydrate in defined medium‡	Turbidity*		
	<i>H. pleuro- pneumoniae</i>	<i>H. parainfluenzae</i> (Boss)	<i>H. parainfluenzae</i> (K-17)
Control.....	0	0	0
Lactate.....	66	55	0
Glycerol.....	0	0	5
D-Glucose.....	109	174	170
Lactose.....	0	0	0
D-Arabinose.....	0	0	0
D-Xylose.....	227	147	265
Maltose.....	100	190	60
Sucrose.....	154	157	0
Mannitol.....	140	140	0
Galactose.....	15	4	4

* Turbidity measured as in Table I.

‡ Carbon source at 0.01 M concentration and 0.02 M HCO₃⁻.

Colonial Morphology.—

As has been mentioned briefly in earlier publications (1, 2), cultures of *H. pleuropneumoniae* derived at autopsy from tissues of swine with PCP yielded two types of colonies of small, plump, Gram-negative bacilli or coccobacilli when cultured on Levinthal agar.

These were: (a) a tiny hard waxy colony which adhered to the platinum loop and was difficult to disperse in broth, and (b) a smooth iridescent colony. The

hard waxy type of colony was the predominant type; the smooth iridescent type of colony was infrequent in some cultures. The smooth iridescent type uniformly emerged in cultures of single colony isolates of the hard waxy type and is of presumed mutational origin. The waxy type colonies increased in size with incubation beyond 18 to 24 hours and became softer in texture and slightly mucoid. A hazy periphery was usually noted in colonies after prolonged incubation of the cultures of the waxy type, and scraping of these colonies from the agar surface revealed a core which extended into the agar.

Capsules were demonstrable in organisms from both colony types after contact with serum of swine convalescent from PCP or serum of rabbits immunized with *H. pleuropneumoniae* of the smooth iridescent type. Capsules were not demonstrable in *H. pleuropneumoniae* after exposure to type specific antisera of *H. influenzae* (types a to f), *H. parainfluenzae* (Boss), or *H. parasuis* (1356).

Interspecific Transformation.—

Interspecific transformation has been used as a tool in the study of genetic relationships among species (or genera) of bacteria. In general, the proportion of cells transformed by a heterologous species DNA is low relative to the proportion transformed by the homologous species DNA. For example, the heterospecific to homospecific transformation ratio is low for *H. influenzae* populations exposed to *H. parainfluenzae* and *H. parasuis* DNA (3); it may approach unity at times, however, when *H. influenzae* populations are exposed to *H. aegyptius* DNA or *H. aegyptius* populations to *H. influenzae* DNA (4). A high degree of genetic homology has been proposed for these latter two species (4). It was of interest, therefore, to compare the relationship of *H. pleuropneumoniae* to other species of *Hemophilus* using interspecific transformation as an index of relatedness.

H. pleuropneumoniae (iridescent type) was virtually incompetent as receptor in transformation studies. The waxy type of culture was not examined as receptor because of the clumping of cells during growth in Levinthal broth. DNA-containing fractions were prepared from streptomycin-resistant mutant populations of both the iridescent and waxy culture types.

The data of Table III are from experiments in which populations of four different species, *H. influenzae*, *H. aegyptius*, *H. parainfluenzae*, and *H. parasuis*, were exposed for 15 minutes to their homologous species DNA and *H. pleuropneumoniae* (iridescent type) DNA, and the number of streptomycin-resistant transformants was measured. The data show that the ratio (expressed as per cent) of the number of cells transformed by the *H. pleuropneumoniae* DNA to the number transformed by the homologous species DNA is low for all recipient populations except *H. parasuis*; a high degree of genetic homology between *H. pleuropneumoniae* and *H. parasuis* is suggested. Similar transformation ratios were obtained when a DNA-containing fraction derived from the waxy colony type of *H. pleuropneumoniae* was used.

Fine Structure.—

Lower power fields of *H. pleuropneumoniae* in the electron microscope show a pleomorphic population of cells ranging in length from 0.5 to 4.0 μ . Two predominant forms are seen:

“healthy” dividing cells (Fig. 1, *H*; Figs. 2 and 3), and involutinal spheroplasts (Fig. 1, *S*; Fig. 4). The healthy cells are generally rod-shaped and their fine structure is similar to that of other Gram-negative bacteria (18). The cell wall (*CW*) consists of a moderately electron-opaque layer about 150 Å wide which is bounded on its innermost surface by a thin dense line (Figs. 1 and 3). An extraneous coat (*EC*) consisting of radially oriented, fuzzy material is adherent to the cell wall (Figs. 1 and 3). Internal to the cell wall and bounding the cytoplasm is a unit membrane (*PM*) about 80 Å wide, consisting of two electron-opaque leaflets enclosing a less dense space (Figs. 2 and 3). This structure is not visible in every cell and is more readily seen where the cell wall is lifted away from the underlying plasma membrane (*PM*) (Fig. 2). The interior of the cell contains many ribosomes (*R*) about 200 Å in width which constitute a wide cortical layer beneath the plasma membrane (Figs. 1 to 3). The remainder of the cell is occupied by nuclear material in the form of bundles of fine, 20 Å wide fibrils (*NF*), which form

TABLE III
Interspecific Transformation within Genus *Hemophilus*; Comparison of Number of Cells Transformed to Streptomycin-Resistance by Homologous “Species” DNA and *H. pleuropneumoniae* DNA

Streptomycin-sensitive recipient, species and strain	Transformants per ml*					<i>H. pleuropneumoniae</i> homo-specific transformation ratio per cent
	Species source of DNA					
	<i>H. influenzae</i>	<i>H. aegyptius</i>	<i>H. parainfluenzae</i>	<i>H. parasuis</i>	<i>H. pleuropneumoniae</i>	
<i>H. influenzae</i> Rd.	2.1×10^6				2.1×10^2	0.01
<i>H. aegyptius</i> 15		4.2×10^6			2.2×10^2	0.05
<i>H. parainfluenzae</i> (Boss)			6.6×10^6		2.4×10^3	0.36
<i>H. parasuis</i> (1356)				5.2×10^2	1.3×10^2	25.

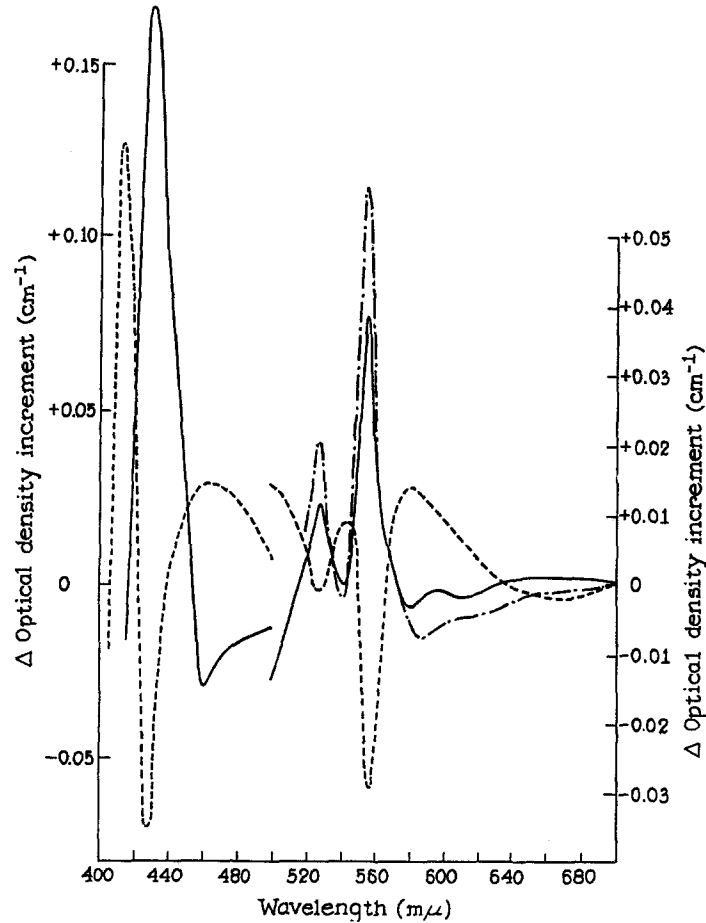
* Approximately 3×10^8 sensitive cells.

a well defined nucleoid in some cells or appear to be arranged as a meandering system of interconnected bundles (Figs. 1 and 2). Chondrioides (mesosomes) or other forms of internal membranes are not observed, though ingrowing division septae (*Se*) are occasionally seen (Fig. 1).

The second cell type seen in section is probably a form of spheroplast (Fig. 4). Here the extraneous coat is generally absent and the cell wall attenuated in thickness. The plasma membrane (*PM*) is visible in most of these cells and consists of a symmetrical unit membrane about 80 Å wide (Fig. 4). The content of ribosomes is decreased or absent and the nuclear material is dispersed as fine, individual threads (*NF*) (Fig. 1). The over-all electron opacity of the cytoplasmic sap is markedly decreased. Many large amorphous electron-opaque masses (*AM*) are scattered throughout the cell and may represent coalesced material resulting from the breakdown of cell components (ribosomes, nuclear material) (Figs. 1 and 4) during the process of involution. Many intermediate forms between these two cell types are seen, and it is probable that in time the more compact typical bacterial form develops into the involutinal spheroplast.

The fine structure of *H. pleuropneumoniae* is similar to that of other members of the *Hemophilus* group, such as *H. influenzae*, *H. aegyptius*, and *H. para-*

influenzae. All the *Hemophilus* species are characterized by pleomorphism at late stages of incubation, and this pleomorphism is correlated with the formation of spheroplast-like involutinal forms with attenuated cell walls.



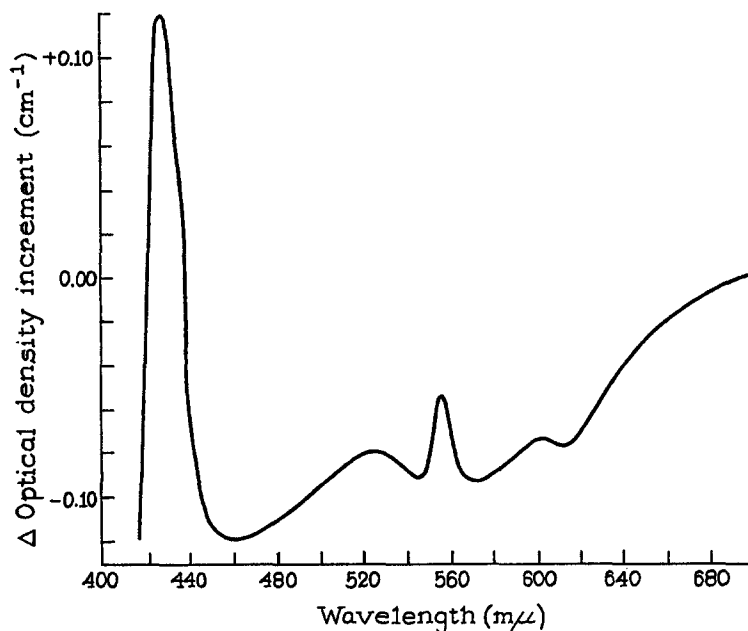
TEXT-FIG. 1. Difference spectra of *H. pleuropneumoniae*. Spectra measured in 0.05 M phosphate buffer pH 7.6 containing 20 per cent (v/v) glycerin at a bacterial density of 10 mg protein/ml. —, DPNH reduced vs. oxidized spectrum. ----, DPNH reduced saturated with carbon monoxide vs. DPNH reduced spectrum. - · - ·, $\text{Na}_2\text{S}_2\text{O}_4$ reduced vs. oxidized spectrum. · · · ·, DPNH reduced vs. oxidized spectrum.

Formation of the Electron Transport System.—

The hemin-requiring species of *Hemophilus* can grow fermentatively without forming a detectable cytochrome system. They can grow anaerobically without added nitrate or in the presence of 0.005 M KCN (17). The hemin-independent species, on the other hand, cannot grow fermentatively and must form an electron transport system in which cytochrome oxidase is

oxidized by oxygen or nitrate. They also form an electron transport system which branches at the oxidase end and can degenerate in the stationary phase with the formation of non-enzymatically reducible cytochrome c_1 (16). This soluble cytochrome has been isolated from all hemin-independent *Hemophilus* species tested (17).

H. pleuropneumoniae cannot grow anaerobically without nitrate or in the presence of cyanide. As is characteristic of *Hemophilus* species, it has the unusual permeability characteristic of reacting with reduced diphosphopyridine nucleotide (DPNH).



TEXT-FIG. 2. Difference spectrum of nitrate reductase of *H. pleuropneumoniae* reduced with 0.005 M DPNH and reoxidized in the presence of 0.02 M KNO_3 . (10 mg bacterial protein/ml)

The difference spectrum of an *H. pleuropneumoniae* population in which respiratory pigments were reduced by DPNH was compared with the same suspension in which respiratory pigments were oxidized. The results are shown in Text-Fig. 1. The three oxidase cytochromes a_1 (maxima at 600 and 424 $m\mu$), a_2 (maximum at 640 $m\mu$), and 0 (maxima at 567, 537, and 414 $m\mu$) are seen in the difference spectrum of *H. pleuropneumoniae* whose reduced respiratory pigments are complexed with carbon monoxide. The large amount of cytochrome c_1 (maxima at 553, 523, and 423 $m\mu$) not reducible with DPNH can be seen in *H. pleuropneumoniae* whose pigments are reduced by $Na_2S_2O_4$. The adsorption maxima of *H. pleuropneumoniae* cytochrome c_1 correspond to those earlier found for *H. parainfluenzae* (Boss) (19). Growth in the presence of nitrate induces the formation of cytochrome a_1 and a larger proportion of this cytochrome, when reduced enzymatically, is reoxidized by NO_3^- than by oxygen in *H. parainfluenzae* (Boss). Text-fig. 2 shows that this is also the case with *H. pleuropneumoniae*.

It is evident from the results obtained that *H. pleuropneumoniae* forms an electron transport system typical of hemin-independent *Hemophilus species*.

DISCUSSION

The causal agent of porcine contagious pleuropneumonia (PCP) has been identified as a member of the *Hemophilus* group; it requires DPN, but not hemin, for growth and has been shown to have certain other growth characteristics in common with two other species of hemin-independent *Hemophilus*, *H. parainfluenzae* and *H. parasuis*. Interspecific transformation studies (Table III) are in support of the assignment of the PCP organism to the *Hemophilus* group. On the premise that the degree of reactivity of donor and recipient DNA is a reflection of homology, it is suggested that this organism may be more closely related to *H. parasuis* than to *H. influenzae*, *H. aegyptius*, or *H. parainfluenzae*. Since only one genetic characteristic (streptomycin resistance) was used as a genetic marker, this suggestion is made with the caution due. Although the data presented establish that the PCP agent belongs to the *Hemophilus* group and suggest that it is closely related to *H. parasuis*, it has been made a species separate from *H. parasuis* on the basis of the marked qualitative difference in the diseases with which the two organisms are etiologically associated as well as certain other qualitative differences in their pathogenicity for swine. The causative agent of PCP has been, as indicated earlier (1), designated *Hemophilus pleuropneumoniae* because of the cardinal lesion that it regularly causes when infecting swine.

The qualitative differences in the pathogenicity of the two organisms for swine, upon which the designation of *H. pleuropneumoniae* as a species separate from *H. parasuis* is based, may be summarized as follows:

1. The clinical entities with which the organisms are causally associated are, as has been pointed out earlier (1), quite separate and easily distinguishable diseases. Swine influenza, with which *H. influenzae suis* is etiologically associated, is a highly contagious but seldom fatal respiratory ailment. The pneumonia in this disease is lobular in distribution and largely limited to the anterior lobes of the lung (20). It is only rarely that this pneumonia is complicated by a concomitant pleuritis. The mortality of both the natural and the experimentally induced disease lies between 2 and 4 per cent, and the size of the infecting inoculum plays little if any role in determining the lethality of the outcome. PCP, with which *H. pleuropneumoniae* is etiologically associated, is, like swine influenza, a contagious respiratory ailment. However, in contrast to swine influenza, the pneumonia of PCP is ordinarily lobar, tends to involve the posterior lobes of the lung primarily, and is always complicated by a concomitant pleuritis. The mortality of the natural outbreak from which our material originally came was about 6.5 per cent (1). The over-all mortality of the experimental disease, however, has approached 50 per cent and is markedly dependent upon the size of the infecting inoculum employed (2).

Upon recovery from swine influenza, the pneumonic lesions resolve rather rapidly

so that by 3 to 4 weeks postinfection, little or nothing other than scant puckered areas of cicatricial tissue mark the former areas of pneumonia. The lungs of swine recovered from PCP, on the other hand, show large hard areas of organizing pneumonia containing necrotic foci and an overlying densely organized pleuritis for at least as long as 14 weeks postinfection.

2. *H. influenzae suis* has in the past, on innumerable occasions, been administered in large dosage intranasally to swine without causing observable illness or pulmonary lesions (21), and recently (2) the particular strain of *H. parasuis* used in the present study has been administered intranasally to swine in enormous doses without effect. Only when the swine influenza virus accompanies the organism does it exhibit significant pathogenicity for swine. In contrast to the complete inability of *H. influenzae suis* acting alone to cause clinical illness or induce pathologic pulmonary changes in swine, *H. pleuropneumoniae* is a very effective pathogen. As has been detailed in a previous paper (2), as few as 100 organisms intranasally have produced the characteristic pleuropneumonia and, though the usually fatal dose is about one-half million, as few as 1400 organisms have been lethal.

3. Not only is *H. influenzae suis* never, when given alone, pathogenic for swine while *H. pleuropneumoniae* is almost always so, but also the contribution that *H. influenzae suis* makes, with the virus, to the pathological manifestations of swine influenza is entirely different from the pathological picture of PCP caused by *H. pleuropneumoniae* alone. The contributions of *H. influenzae suis* to the pathological manifestations of swine influenza appear to be two fold. First, the gross extent of the pneumonic lesions is greater in swine infected with both the virus and *H. influenzae suis* than it is in animals infected with the virus alone (22). Secondly, the viral histopathology is modified by the concomitant bacterial infection in that polymorphonuclear leucocytes are much more numerous than they are in the purely virus pneumonias. This increased polymorphonuclear reaction is most prominently evident in the small bronchi and bronchioles and in the alveoli adjacent to them. In contrast, *H. pleuropneumoniae* appears to call forth in the lung primarily a lymphocytic cellular reaction and although, as in swine influenza, the small bronchi, bronchioles, and the alveoli surrounding them are involved, the primary inflammatory reaction appears to be in and about the lymph channels of the pleurae and the interlobular septa. The pneumonia, as manifested by cellular exudation, seems to involve first the alveoli adjacent to the lymph channels, either below the pleural surfaces or along the interlobular septa (1). The two organisms, therefore, differ markedly in the histopathological reactions they cause in the separate diseases in which they are involved.

The qualitative differences in the types of disease with which they are etiologically associated, together with the differing histopathological reactions elicited in the swine respiratory tract by the two organisms are believed sufficient to justify the establishment of *H. pleuropneumoniae* as a species separate from *H. parasuis*. In addition, the complete failure of pathogenicity of *H. parasuis*, except in the presence of a concomitantly infecting virus, and the marked pathogenicity of *H. pleuropneumoniae* as an unassisted infective agent rather strikingly demonstrate intrinsic qualitative differences in the disease-producing capabilities of the two organisms.

When we began our studies of PCP, we were not aware that a disease of its character had ever before been described or investigated. Recently, however, our attention has been called to work by Pattison, Howell, and Elliot (23), and, Matthews and Pattison (24) on a respiratory ailment in British swine that, though much milder and more difficult to transmit experimentally than PCP, nevertheless reminds us of a benign version of our Argentine disease. The primary pathological lesion of the British disease is a pneumonia associated with pleuritis and the causative agent is a DPN-dependent *Hemophilus* which has been designated *Haemophilus para-influenzae* (24). This *Hemophilus*, when administered intratracheally in relatively large dosage, induces a pleuropneumonia that is evidently, from its description, much milder than that caused by our Argentine *Hemophilus* intranasally. However, the pathology of the condition, both gross and histologic, is qualitatively similar to that of the Argentine disease. The concomitant presence of hog cholera virus seemingly enhances the pathogenicity of *H. para-influenzae* and the disease resulting from this mixed viral and bacterial infection then approaches more nearly that of PCP in its clinical severity. However, the rapidly fatal fulminating infections that we have seen in swine infected with our *H. pleuropneumoniae* were not observed by the English workers even when they administered hog cholera virus with their organism. A relationship between the British swine pleuropneumonia and PCP cannot be positively established from the information available, but it seems entirely possible that they may be quite similar diseases, with similar causative agents, differing only quantitatively in their pathological and clinical severity.

SUMMARY

Hemophilus pleuropneumoniae, the causative agent of porcine contagious pleuropneumonia (PCP) is an encapsulated organism that has the metabolic features of the parainfluenza group of *Hemophilus* in that it requires DPN but not hemin for growth. Its formation of nitrate reductase cytochrome a_1 and non-physiologically reducible cytochrome c_1 in the stationary phase, together with its requirement of electron transport through oxidases for growth are typical of non-hemin-requiring *Hemophilus* species. It has the closest genetic homology, judged from the capacity of its DNA to induce transformation to streptomycin resistance, with *H. parasuis* but can be differentiated from this organism on the basis of its growth in defined medium and its marked and characteristic pathogenicity for swine.

BIBLIOGRAPHY

1. Shope, R. E., Porcine contagious pleuropneumonia. I. Experimental transmission, etiology, and pathology, *J. Exp. Med.*, 1964, **119**, 357.
2. Shope, R. E., White, D. C., and Leidy, G., Porcine contagious pleuropneumonia.

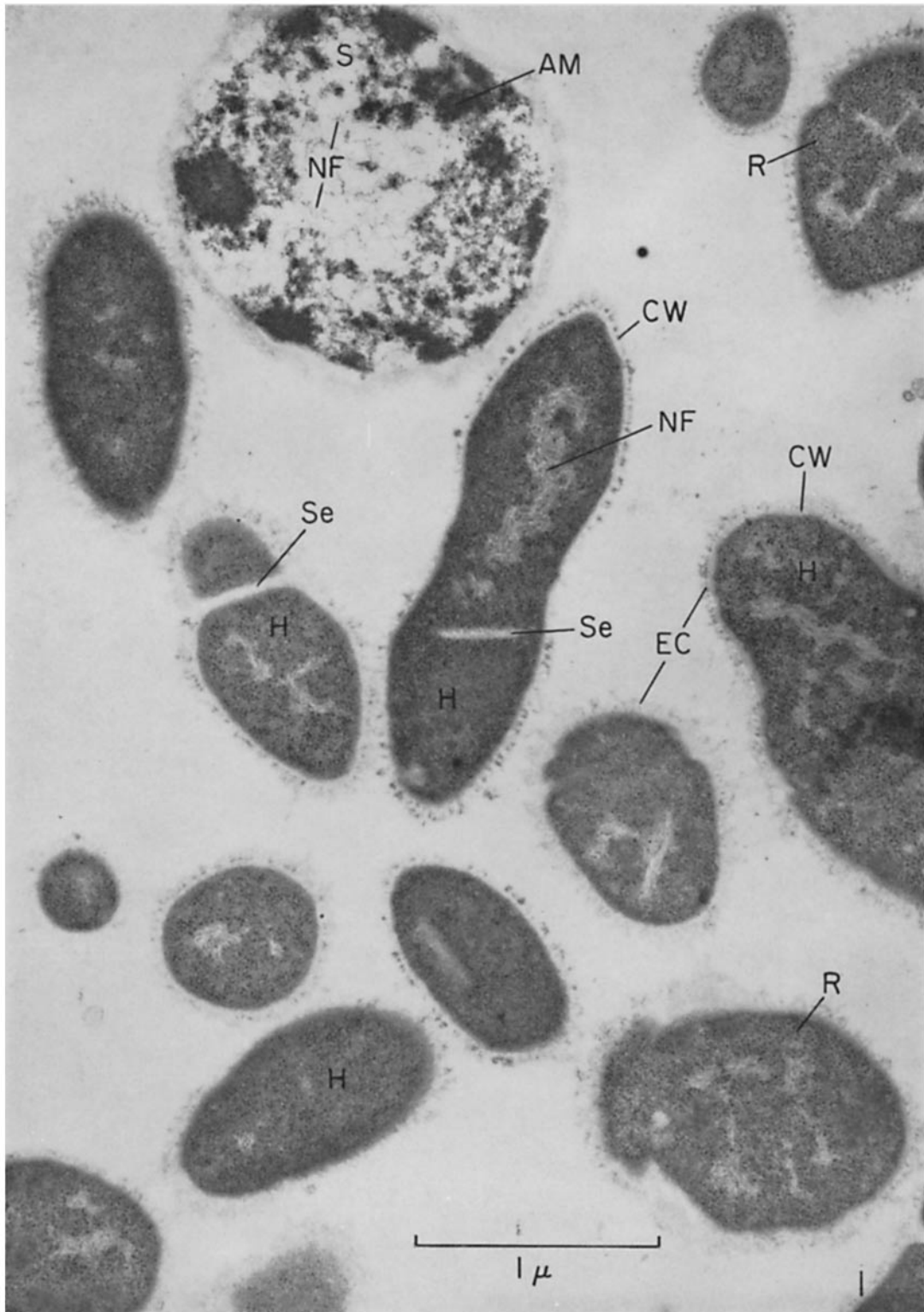
- II. Studies of the pathogenicity of the etiological agent, *Hemophilus pleuropneumoniae*, *J. Exp. Med.*, 1964, **119**, 369.
3. Leidy, G., Hahn, E., and Alexander, H. E., On the specificity of the desoxyribonucleic acid which induces streptomycin resistance in *Hemophilus*, *J. Exp. Med.*, 1956, **104**, 305.
 4. Leidy, G. E., Hahn, E., and Alexander, H. E. Interspecific transformation of *Hemophilus*. A possible index of relationship between *H. influenzae* and *H. aegyptius*, *Proc. Soc. Exp. Biol. and Med.*, 1959, **102**, 86.
 5. White, D. C., and Granick, S., Hemin biosynthesis in *Haemophilus*, *J. Bact.*, 1963, **85**, 842.
 6. Lewis, P. A., and Shope, R. E., Swine influenza. II. A hemophilic bacillus from the respiratory tract of infected swine, *J. Exp. Med.*, 1931, **54**, 361.
 7. Biberstein, E. L., and Gills, M., Catalase activity of *Haemophilus* species grown with graded amounts of hemin, *J. Bact.*, 1961, **81**, 380.
 8. White, D. C., and Smith, L., Hematin enzymes of *Hemophilus parainfluenzae*, *J. Biol. Chem.*, 1962, **237**, 1332.
 9. Herbst, E. J., and Snell, E. E., The nutritional requirements of *Hemophilus parainfluenzae* 7901, *J. Bact.*, 1949, **58**, 379.
 10. Leidy, G., Jaffe, I., and Alexander, H. E., Emergence of competence (for transformation) of three *Hemophilus* species in a chemically defined environment, *Proc. Soc. Exp. Biol. and Med.*, 1962, **111**, 725.
 11. Alexander, H. E., The *Haemophilus* group, Bacterial and Mycotic Infections of Man, Philadelphia, J. B. Lippincott Co., 3rd edition, 1958, 470.
 12. Alexander, H. E., and Leidy, G., Determination of inherited traits of *H. influenzae* by desoxyribonucleic acid fractions isolated from type-specific cells, *J. Exp. Med.*, 1951, **93**, 345.
 13. Ryter, A., and Kellenberger, D., Etude au microscope électronique de plasmas contenant de l'acide désoxyribonucleique, *Z. Naturforsch.*, 1958, **13 b**, 597.
 14. Luft, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
 15. Karnovsky, M. J., Simple methods for "staining with lead" at high pH in electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 729.
 16. White, D. C., Cytochrome and catalase patterns during growth of *Haemophilus parainfluenzae*, *J. Bact.*, 1962, **83**, 851.
 17. White, D. C., Respiratory systems in the hemin-requiring *Haemophilus* species, *J. Bact.* 1963, **85**, 84.
 18. Kellenberger, E., and Ryter, A., Cell wall and cytoplasmic membrane of *Escherichia coli*, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 323.
 19. Smith, L., and White, D. C., Structure of the respiratory chain system as indicated by studies with *Hemophilus parainfluenzae*, *J. Biol. Chem.*, 1962, **237**, 1337.
 20. Shope, R. E., Swine influenza. I. Experimental transmission and pathology, *J. Exp. Med.*, 1931, **54**, 349.
 21. Shope, R. E., The influenzas of swine and man, *Harvey Lectures*, 1935-36, **31**, 183.
 22. Shope, R. E., Swine influenza. III. Filtration experiments and etiology, *J. Exp. Med.*, 1931, **54**, 373.

23. Pattison, I. H., Howell, D. G., and Elliot, J., A *Haemophilus*-like organism isolated from pig lung and the associated pneumonic lesions, *J. Comp. Path. and Therap.*, 1957, **67**, 320.
24. Matthews, P. R. J., and Pattison, I. H., The identification of a *Haemophilus*-like organism associated with pneumonia and pleurisy in the pig, *J. Comp. Path. and Therap.*, 1961, **71**, 44.

EXPLANATION OF PLATES

PLATE 1

FIG. 1. Low-power electron micrograph of a thin section of *H. pleuropneumoniae*. Two main cell types, "healthy" dividing cells (*H*), and spheroplasts (*S*) are seen. *NF*, nuclear fibrils; *CW*, cell wall; *EC*, extraneous coat; *R*, ribosomes; *Se*, division septum; *AM*, amorphous mass. $\times 37,000$.



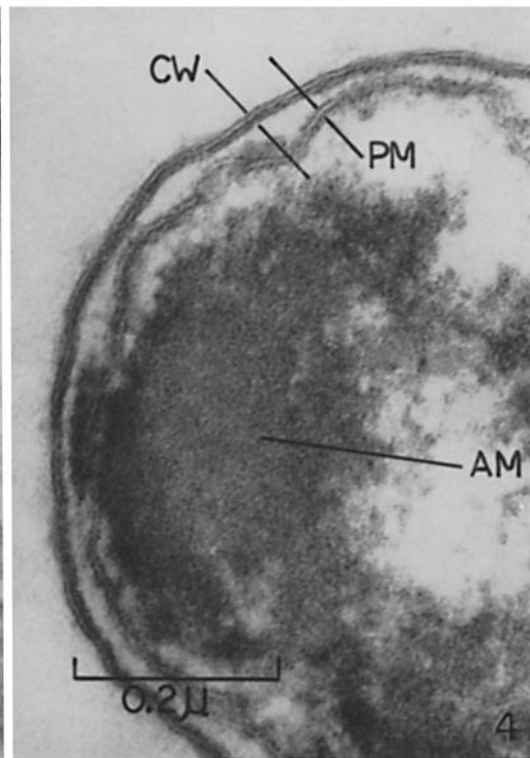
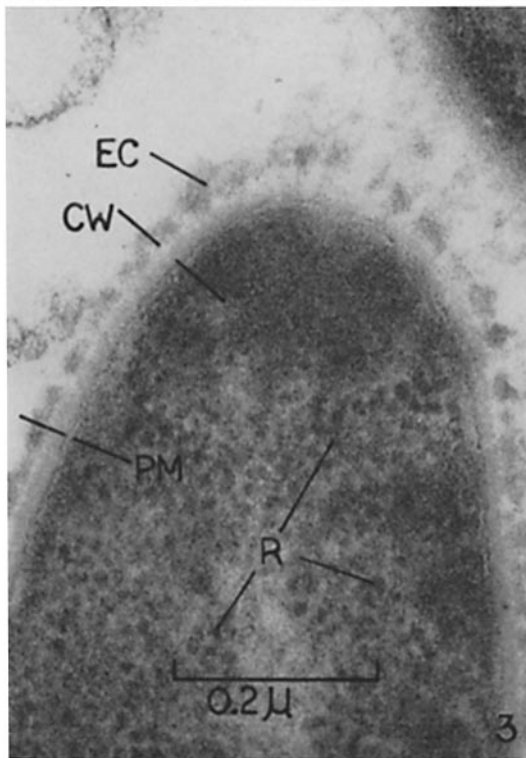
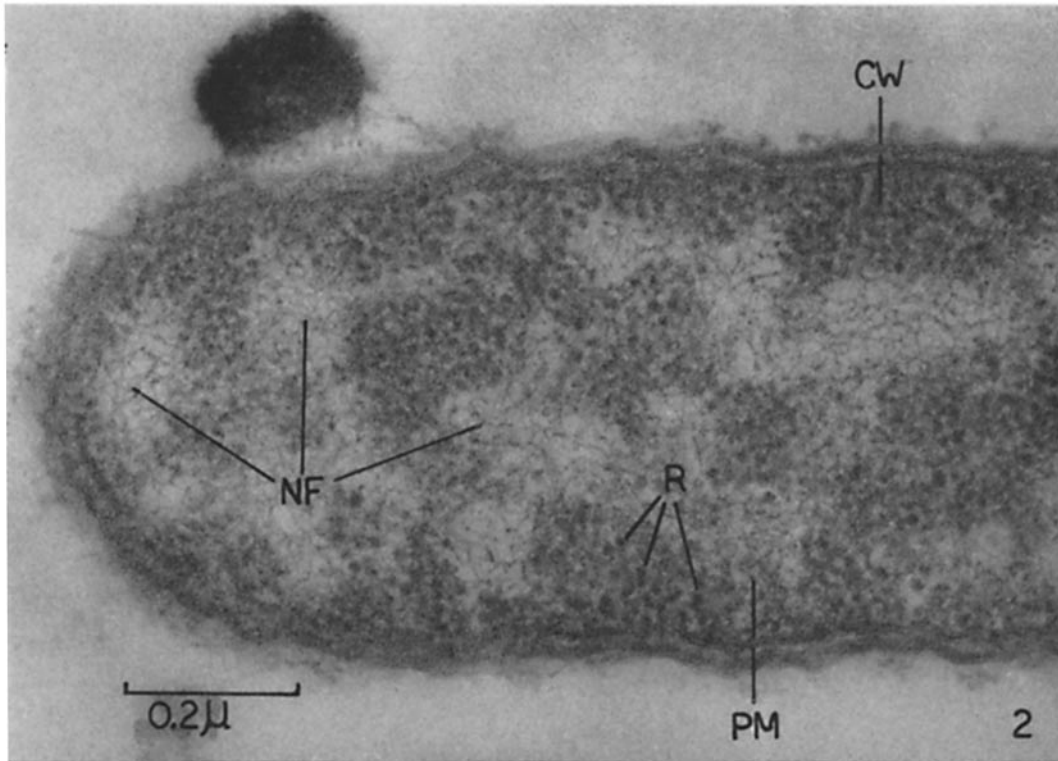
(White *et al.*: Porcine contagious pleuropneumonia. III)

PLATE 2

FIG. 2. Healthy form of *H. pleuropneumoniae*. *CW*, cell wall; *R*, ribosomes; *NF*, nuclear fibrils; *PM*, plasma membrane. $\times 115,000$.

FIG. 3. Portion of healthy cell illustrating extraneous coat (*EC*), cell wall (*CW*), plasma membrane (*PM*), and ribosomes (*R*). $\times 135,000$.

FIG. 4. Spheroplast form of *H. pleuropneumoniae*. *CW*, cell wall; *PM*, plasma membrane; *AM*, amorphous mass. $\times 135,000$.



(White *et al.*: Porcine contagious pleuropneumonia. III)