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Scope and Method of Study: This report represents an attempt to review what is presently known about PPLO as concerns their relationship to other life forms and their pathogenicity. A selected portion of the scientific literature on this subject is cited.

Findings and Conclusions: PPLO are thought to be related to bacteria, probably through their L-forms. Our knowledge of their morphology is still incomplete. PPLO have been found to be the cause of diseases of both animals and men. A great deal of controversy concerning the pathogenicity of PPLO still exists.

ADVISER'S APPROVAL L. Herbert Bruneau

PLEUROPNEUMONIA-LIKE ORGANISMS

By

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PREFACE AND ACKNOWLEDGMENTS

This report is an attempt to bring together some of the more important opinions and facts concerning Pleuropneumonia-like organisms (PPLO) with regard to their relationship to other forms of life and their pathogenicity. This report is not a complete representation of either of these facets, and is not intended to be so. I hope to point out however, what, after reading a certain amount of the literature dealing with pleuropneumonia-like organisms, I find to be areas of disagreement and confusion.

Many more studies are available in the literature than I have cited. In dealing with the pathogenicity of these organisms in animals for example, I have concentrated mainly on studies of avian PPLO. A great deal of work has been done in this area because of its economic importance which in turn amply highlights the problems and knowledge in the field of PPLO as animal pathogens.

Due to the controversial nature of many of the aspects of PPLO, it would be wise for the reader who does not already have a certain amount of knowledge concerning PPLO to read this paper and consider its contents with an open mind, carefully analysing the information presented.

I would like to express my thanks to Dr. Herbert L. Bruneau, my advisor, for his help on this report and all through my graduate work. Also very deserving of my thanks is Dr. Edward Grula, whose helpful criticism and technical assistance were a great help to me. His patience with my errors and generous assistance at all times were invaluable to me.

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CHAPTER I

INTRODUCTION

In the last several years, many investigations have been made to determine the relationship of pleuropneumonia-like organisms (PPLO) to other forms of life, and to determine their significance as pathogens of animals and men. In the course of this work, many unique and sometimes frustrating problems peculiar to the study of these organisms have arisen. Many of these problems remain unsolved.

The first question which demands an answer is what are PPLO? Are they related to bacteria or viruses? Are they some aberrant form of bacterial growth which has become established, and which has now evolved to the point where they are a stable and separate life form? What are the reasons for the controversy in this area? Many prominent workers have attempted to answer these questions, and the work of several investigators relating to this matter will be reviewed in the following chapter.

The problem of the pathogenicity of these organisms is extremely important because these organisms have been implicated as the probable cause of several economically important animal diseases. In fact, much of what we now know about PPLO has been derived from studies of PPLO as causes of these diseases in animals which have economic importance. PPLO have also been cultured from humans, and are suspected to be the causal factor of several human diseases. In attempting to determine the pathogenicity of PPLO in these different instances, investigators have found

that again, they are faced with unexpected difficulties. Because of the difficulty of culturing these organisms and accurately identifying different types, it has proven extremely difficult, especially for the earlier investigators, to fulfill Koch's postulates.

In the following chapters, the experiments and results of several investigators will be presented which will more clearly illustrate the extent of the difficulties faced in this area. Special attention will be given to the reasons for some of the controversy and to several of the outstanding problems in this field.

CHAPTER II

PPLO IN RELATION TO OTHER LIFE FORMS

Before attempting to achieve some concept of the relationship of PPLO to other forms of life, it would be well for the reader to be aware of the morphology of these organisms. Although a large number of studies have been made concerning the morphology of PPLO, a great deal of controversy remains. Two major concepts of PPLO morphology have evolved over the years which are almost directly opposed to each other.

One of these important concepts is clearly described by Freundt (1960) in an article in which he gives a description of the development of the organism of bovine pleuropneumonia. The methods used in observing these organisms is of great importance in answering the question of their morphology. The method of observation used by Freundt (1960) involved the use of a square of agar containing PPLO colonies upon which several cotton fibers and a drop of water were placed. This is then covered with a coverslip.

Using this technique, the following developmental sequence can be observed. The beginning of development is an elementary body which has a diameter of approximately 250 to 300 millimicrons. Extending from this elementary body are found one or more very fine branching filaments which are reported by Freundt (1960) as being optically homogeneous. A

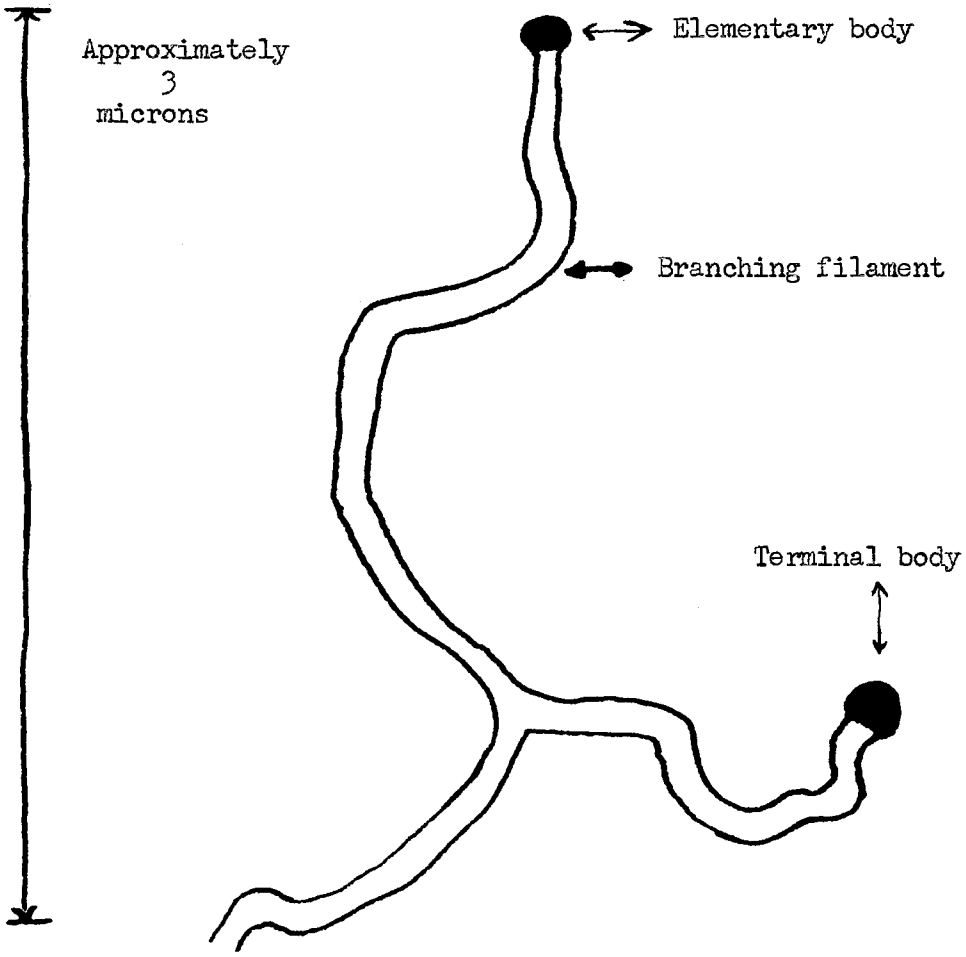
structure of approximately the same size as the elementary body which is referred to as a terminal body is always found at the end of these filaments. The function of the terminal body is that of a center of growth from which more filaments will arise. Freundt also states that, "A true lateral branching characterized by the apparently coenocytic outgrowth of lateral branches from the stem filament, occurs fairly frequently."

Next in the sequence of growth is the development of chains which begins when the filaments are seen to contain uniform spherical bodies that appear to be highly refractile. Constrictions begin to appear in the filament which serve to separate the spherical bodies and divide the filament into a series of coccoid bodies which, according to Freundt, are new elementary bodies. Drawings of some of the electronmicrographs presented by Freundt showing stages in this sequence are shown on the next page, (Freundt, 1960).

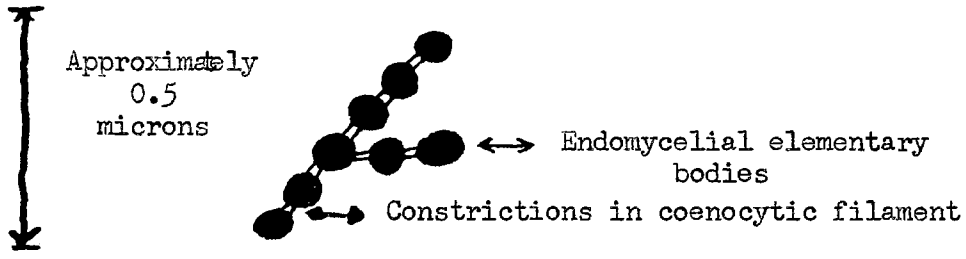
The opposing view concerning the morphology of these organisms is based upon observations of PPLO using a different technique. According to Liebermeister (1960), a confusing picture of the morphology and multiplication of these organisms has been presented over the years mainly because of two things. First of all, these organisms are inherently difficult to observe because of their small size. Under the light microscope it is difficult to observe these organisms. The size of the smallest object which can be resolved by the light microscope is approximately 0.2 microns. PPLO are often as small as 250 millimicrons.

TABLE I

A BRANCHING FILAMENT OF MYCOPLASMA MYCOIDES



A filament with
endomycelial
elementary bodies



(Freundt, 1960)

Second, although it is well known by investigators in the field that these organisms do not possess a cell wall, and hence are very fragile and easily distorted, many investigators still use methods of observation which probably alter the appearance of these organisms.

Liebermeister used the techniques of agar fixation and agar filtration, which he claims does not distort the appearance of PPLO. He observes a simpler morphology, which from the electron micrographs he presents, and from his discussion, appears to include bodies which vary in size and shape, the predominent shape being more elliptic than round. His pictures do not show the presence of any filamentous structures. As a sort of control, Liebermeister also compares his observations using the electron microscope to observations using similar techniques with the light microscope. He explains the presence of filaments observed by other workers as artifacts due to mechanical deformation of these fragile organisms. He contends that the pressures produced by lowering the objective into the oil on the coverslip is sufficient to produce fluid pressures which cause distortion of these organisms. Relative to multiplication of these organisms, he merely states that they seem to grow by a sort of sprouting, (Liebermeister 1960).

In answering the objections to the techniques he uses, Freundt (1960) brings up what appears to be an important consideration. While admitting that his technique does cause fluid pressures to arise and flow along the strands of cotton placed under the coverslip, he asks the question, why, if these pressures are all exerted in one direction, that branching filaments are seen. How are these branches formed by pressures that flow in only one direction, (Freundt, 1960)?

The question of the morphology of these organisms is complex and difficult to solve. It seems that better methods of observing these organisms will be needed. Until a technique of observing PPLO is developed which is reliable, the controversy will not be settled.

Relation to Bacteria

The answer to the question as to the relationship of PPLO to other life forms is important for many reasons. Since, as will be shown later, these organisms are pathogens of both animals and humans, it is important that we have as clear an understanding as possible of the nature of these organisms. What are these organisms, and where do they belong in the evolution of life? Our ideas concerning these organisms have continually changed as we have learned more and more about them. At present, the major efforts in research along this line are directed towards determining the relationship of PPLO to bacteria.

One of the most important factors leading to the development of the concept that bacteria and PPLO are somehow related concerns the L-forms of bacteria. The L-forms of bacteria can be induced to form from normal bacteria by growing the bacteria in the presence of penicillin or some other antibiotic which interferes with the synthesis of rigid cell wall material, (Lederberg, 1956). Dienes (1960),presents a table which gives a fairly good idea of the similarities and dissimilarities of PPLO and L-forms.

Many papers have been written concerning attempts to establish the relationship between PPLO, L-forms, and bacteria. The following studies are representative of some of this work.

TABLE II

SIMILARITIES BETWEEN PPLO AND L-FORMS OF BACTERIA

Both are simple organisms without apparent internal structure.

Physical properties such as softness, mechanical fragility, and instability in distilled water indicate the lack of a rigid cell wall.

Cultures of both contain organisms varying in size from small granules to large round bodies.

Agar colonies of both have characteristic appearance and structure.

Response to antibiotics of both, especially to penicillin, is similar.

Inhibition of growth by antibodies in the absence of complement occurs in both types of organisms.

DISSIMILARITIES

L-forms are not well adapted to grow on any of our present media and usually grow under narrowly defined physical conditions. Few of the transferred organisms produce progeny.

The colonies and the individual organisms in them are usually larger than those of PPLO.

L-forms have been observed only in the laboratory, not as independent organisms in nature.

Many strains of PPLO grow as well as bacteria on various media. The cultures are often less pleomorphic than those of L-forms. In the culture of some strains, long branching filaments develop.

(Dienes, 1960)

In 1954, McKay and Taylor report the reversion of what they had termed PPLO to a Gram-variable rod. The PPLO had been cultured in egg, and was associated with such avian diseases as chronic respiratory disease and infectious sinusitis of turkeys, (McKay and Taylor, 1954). McKay and Truscott (1960) later present data indicating the spontaneous reversions of avian PPLO to Hemophilus gallinarum. They also report the reversion of avian PPLO caused by saline extracts, and the reversion of PPLO to Hemophilus gallinarum caused by the addition of DNA extracts from

Hemophilus gallinarum. Their reversion experiment involving the DNA extracts is graphically shown on the next page.

McKay and Truscott state that their experiments do not prove that PPLO and L-forms of bacteria are the same. The authors also contend that more attention should be given to bacterial genetics in the solution of this problem. According to McKay and Truscott,

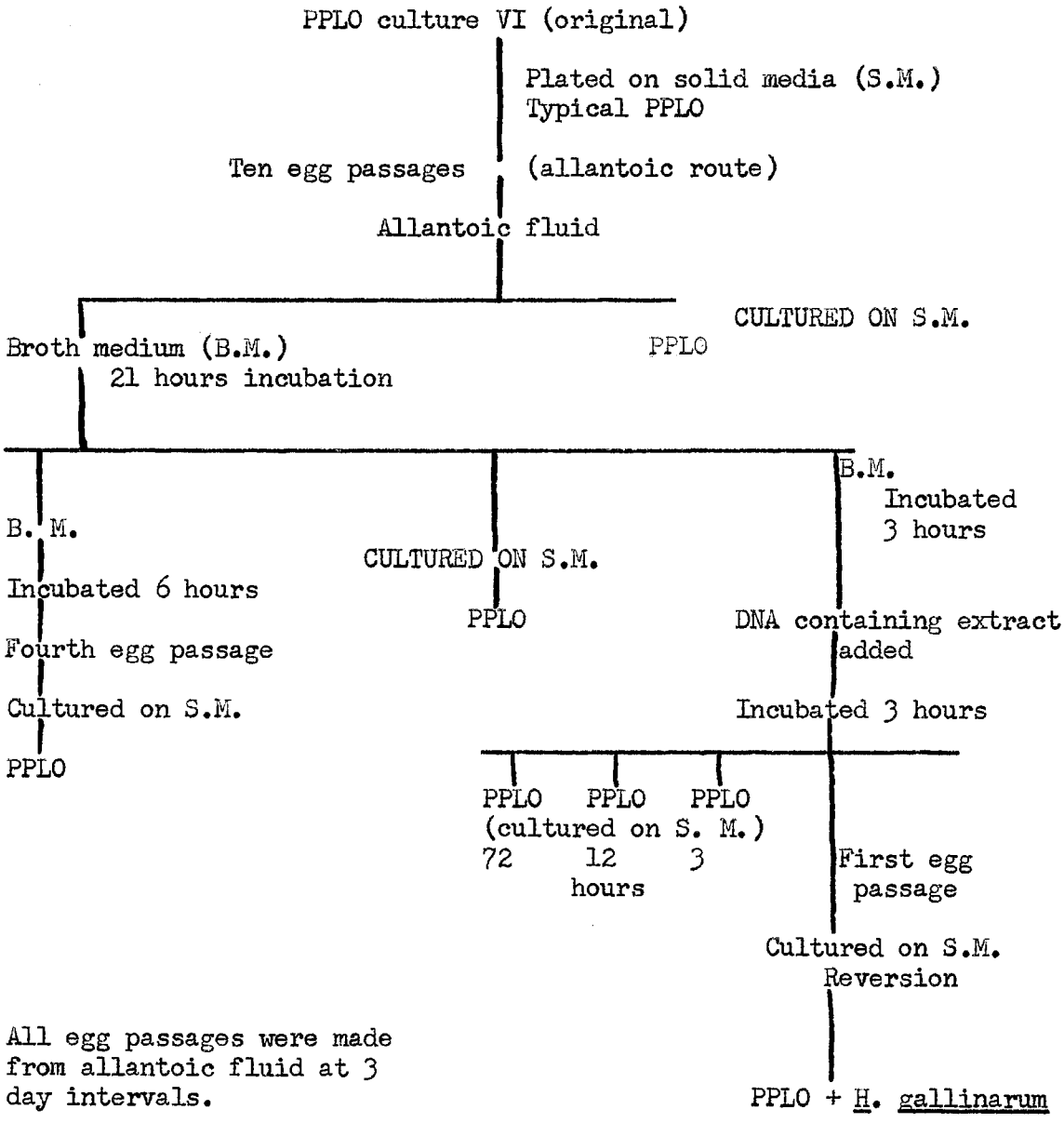
> "Binding proof to establish the relation of PPLO to bacteria will have to consist of the reversion of a classic strain of PPLO to a bacterium, reliably establishing the relationship of each, and finally producing the PPLO from the bacterium," (McKay and Truscott, 1960).

Another report concerning the relationship of L-forms and bacteria was presented by Phyllis Pease in 1962. In this experiment, a corynebacterium of vaginal origin was cultured on Difco PPLO medium upon which was placed a piece of filter paper which had been soaked in a solution of penicillin. After two days, colonies of L-forms appeared. These L-form colonies proved to be unstable, however, when attempts were made to subculture them upon the same medium, but without penicillin, these organisms reverted to a form of corynebacterium. When subculturing was attempted on media containing penicillin, these organisms did not usually survive beyond the second subculture.

In some cases however, subcultures of these pure L-forms gave rise to a filamentous type of organism which was not affected by subsequent subculturing on a medium not affected by subsequent subculturing on a medium not containing penicillin, and which resembled Streptobacillus moniliformis. The author ran serological tests comparing the original corynebacterium, Stroptobacillus moniliformis, and a human strain of PPLO, designated as Mycoplasma hominis Type 1. She interpreted serological findings as indicating a correlation between these organisms. The author's

TABLE III

REVERSION OF PPLO



(McKay and Truscott, 1960)

interpretation of these data is well represented by her statement that,

> "The purpose of this paper is to show that Streptobacillus moniliformis represents a semi-stable form intermediate between the L-stage and the Gram-positive bacilliary form of a corynebacterium," (Pease, 1962).

A report which clears up some of the confusion in this area and which should provide a better background from which to interpret the data presented in reversion experiments was published by H. H. Martin in 1964. Although not dealing with PPLO directly, the author does present valuable information concerning L-forms which must be clearly understood before an overall concept can be formed.

In his work, Martin studied the difference in the cell walls of Proteus and various L-forms produced by the use of antibiotics. The author makes the distinction that spheroplast L-forms are organisms which retain a wall of mucopolymer material. Organisms which have lost all traces of cell wall material, he calls protoplast L-forms. Some of the L-forms studied by the author were unstable; that is, when placed upon media not containing penicillin, they reverted to normal Proteus-like organisms. Others however, which did not revert to normal Proteus-like organisms were found to develop in some cases from the unstable L-forms. The unstable forms retain a mucopolymer cell wall, but the mucopolymer seems to lack organization into a rigid structure. Concerning this fact and its probable cause, the author states,

> "It is significant that mucopolymer containing stable L-forms arise from the particular type of unstable L-form occurring in Proteus. In the latter organisms the consequences of penicillin action observed in other bacteria, namely inhibition of mucopolymer synthesis and autolytic removal of mucopolymer from cell walls are definitely not operative. Here the penicillin seems to act more specifically, presumably by preventing the formation of certain cross linkages within the mucopolymer which are indispensable for the establishment of shape and mechanical stability," (H. H. Martin, 1965).

In this study, the author worked with both mucopolymer synthesizing stable L-forms and with stable L-forms which did not produce any mucopolymer. The author contends that the problem which now must be solved is how is it possible that the reversible condition caused by the penicillin can become established as a permanent hereditary trait.

What can we conclude from the data presented by these several authors dealing with the relationship of PPLO to other life forms? A quick review may bring things more clearly into focus.

PPLO and L-forms resemble each other in several ways. Their colonial and individual morphology is similar, and neither has a rigid cell wall, (Dienes, 1960). McKay and Truscott report a carefully controlled experiment in which an avian PPLO seems to have reverted to Hemophilus gallinarum. Pease presents data which she claims shows that Streptobacillus moniliformis is a transitional element between a corynebacterium and its L-stage. Finally, Martin shows the differences between the cell walls of several organisms ranging from normal Proteus which has a cell wall containing a rigid mucopolymer to a protoplast L-form which has no mucopolymer at all.

The implication of these data seems to be that PPLO are somehow related to bacteria and that the different L-stages in some way form the connecting link. From an evolutionary point of view, hypothesizing now that PPLO have evolved from bacteria and that the unstable and stable L-forms represent possible intermediaries in this development, how could we partially explain our hypothesis in terms of the data we have before us?

According to the data presented by Hardin, it is possible to induce the formation of unstable L-forms of Proteus which at times will in turn give rise to stable L-forms. Stable L-forms range from organisms which have a mucopolymer cell wall which lacks rigidity to organisms completely lacking any mucopolymer material. The character of the cell walls or the lack of cell walls has been shown in these organisms to be a permanent inheritable characteristic. Hamilton (1965) reports that O. E. Landman, Antoinette Ryter, and R. E. Knolt have discovered that L-forms studied by electronmicrographs have no mesosomes. It seems that treatment with lysozyme somehow causes the mesosomes to be lost by eversion. In their studies, these authors are reported to have connected mesosomes to the formation of cell wall and septum, (Hamilton, 1965). These L-forms resemble PPLO in several respects, but in general, according to Dienes (1960), are larger. In order to satisfy the hypothesis, we must now assume that stable L-forms have occurred in nature, (a phenomenon not yet recorded) and that these stable L-forms in some way undergo a transition which is genetically stable, to PPLO. As yet, evidence for such a transition is lacking.

Conversely, it is possible to hypothesize that the opposite is true, that is, that bacteria have evolved from PPLO. Using the same relationships used in explaining the original hypothesis, but in reverse order, it might be possible to explain this second hypothesis. Here it should be remembered that McKay and Truscott have presented data which includes the reversion of a PPLO to a bacterium, thus providing the possibility that such a change could have occurred in nature. Admittedly, there are huge gaps in the explanations of both these hypotheses.

Finally, in considering the relationship of PPLO to bacteria, is it necessary that we consider that one has evolved from the other? Could it be possible that PPLO and bacteria had a common progenitor which would explain why these organisms have features which are similar in some instances but unlike in others? Is this why these organisms are so closely related that it is possible for one to revert to the other by passing through such transitional stages as the L-form of bacteria? Definitely, it would be a great help if we knew the answers to these questions. For the time being however, we must carefully, though arbitrarily define each of these organisms in order that research will be facilitated and that confusion be kept to a minimum.

CHAPTER III

ANIMAL PATHOGENICITY

Many difficulties have arisen in attempts by investigators to determine the true relationship of PPLO to certain diseases of animals. Most of the work in this field has been done since 1938. A large amount of the literature on this subject deals with studies of PPLO involved in avian diseases. This chapter will deal mainly with studies of the pathogenicity of avian PPLO due to the fact that these studies provide a fairly good overall view of the problems involved. It should be mentioned however, that PPLO have been implicated in diseases of mice (Sabin, 1938), goats (Shirlaw, 1949), pigs (Lecce, 1960), and numerous other animals. Several of these diseases are listed in TABLE IV on the following page.

The problem of PPLO as pathogens of other animals besides cattle and sheep began in 1938, when Sabin, while experimenting with toxoplasma in mice, discovered another agent which had the ability to cause central nervous system disease in mice. When he attempted to grow this agent on ordinary media he failed, and therefore could not immediately identify it. (Sabin, 1938). Later that same year, Sabin reports that he was able to grow the organism, and identified it as a PPLO. (Sabin, 1938).

Eleven years later, Shirlaw (1949) reported a study which established a PPLO as the cause of pleuropneumonia of goats. He also concluded that the possibility that the disease is air borne is very great,

TABLE IV

ANIMAL DISEASES INVOLVING PPLO

1. Pleuropneumonia of goats (Shirlaw, 1949).
2. Bovine Pleuropneumonia (Nocard and Roux, 1898).
3. Porcine polyserositis (Lecce, 1960).
4. Chronic respiratory disease of chickens (Markham and Wong, 1952).
5. Infectious sinusitis of turkeys (Markham and Wong, 1952).
6. Neurolytic symptoms in mice (Sabin, 1938).
7. Arthritis of goats (Cordy and Adler, 1960).
8. Infectious agalactia of sheep and goats (Bridre and Donatien, 1925).
9. Bronchopneumonia in rats (Klieneberger and Staeben, 1937).

since he found that contact was not necessary between diseased and healthy goats in order for the disease to be spread. He also found that at least one month after the last fatal case, the infective agent could still be viable in a shed, (Shirlaw, 1949).

Two of the most important avian diseases which seem to involve PPLO are chronic respiratory disease of chickens and infectious sinusitis of turkeys. The relationship of these two diseases were studied by Markham and Wong, (1952). In 1951, the authors obtained strains of the agents of chronic respiratory disease of chickens and of infectious sinusitis of turkeys from several prominent laboratories. First of all, a number of turkeys were inoculated with the agent of infectious sinusitis. In less than ten days, three out of four turkeys inoculated showed typical symptoms including swollen sinuses. Next, turkeys were inoculated with the agent of chronic respiratory disease. Inoculation of this agent produced swollen sinuses similar to those observed in the first part of

the experiment. The authors also contend that the agents of chronic respiratory disease and infectious sinusitis are morphologically similar, (Markham and Wong, 1952).

From this, it would seem that the two diseases are closely related. Because of the staining characteristics of these agents, their reaction of antibiotics, and studies of their morphology, these authors report that the agents of chronic respiratory disease and infectious sinusitis are probably PPLO, (Markham and Wong, 1952).

As further studies were made, several investigators began to discover that they could not always satisfy Koch's postulates when dealing with a PPLO which they found to be connected with certain diseases. For example, Nelson found that upon injection of a suspect PPLO alone into the cranial region of mice, no symptoms were visible, and the level of PPLO in the brain was low. The situation became further complicated when he found that he could produce the disease by injecting both the PPLO he was working with and the virus of murine hepatitus into the brain at the same time, (Nelson, 1957). From this report it seems that at times, the presence of another organism can cause PPLO to become more virulent.

The next report which actually precedes the latter by a year deals with an avian PPLO, and illustrates the occurrence of a phenomenon opposite to that reported in the previous study. Gross while working with E. coli and the PPLO agent of chronic respiratory disease found that,

> "The lesions of pericarditis, perihepatitis, aerosacculitis, salpingitis, and uvietis which are seen in field cases of air sac disease alone, or as complications of chronic respiratory disease, were reproduced by injecting Eschrichia coli alone or with the chronic respiratory disease agent into the air sacs of chickens and turkeys, (Gross, 1956).

Gross also found that the chronic respiratory disease agent increased the pathogenicity of Eschrichia coli when both were injected, (Gross, 1956).

So now we have a report stating that the presence of a PPLO can contribute to the pathogenicity of another organism. In a later report, Gross (1957) explains that certain conditions caused by Eschrichia coli are almost always found in association with an infection of PPLO. It seems that the PPLO infection is prerequisite to the invasion of the respiratory tract by Eschrichia coli, (Gross, 1957).

Probably the most confusing problem faced by investigators attempting to establish PPLO as the cause of chronic respiratory disease and infectious sinusitis is illustrated by Fabricant, (1960), in a review of the problem in which he states,

> "Sometimes it was found that PPLO isolated from cases of chronic respiratory disease failed to produce symptoms or lesions of chronic respiratory disease, even when inoculated into the respiratory tract of susceptible turkeys. In other cases, it was found that the disease could be transmitted with fresh exudates containing PPLO, egg propagated strains of PPLO, or even PPLO propagated on culture media for a few passages. When some of the organisms were carried for several passages on culture media, there was a rapid loss in the pathogenicity of the PPLO. On the other hand, some PPLO cultures retained a high degree of pathogenicity even after many passages in culture media.

A partial explanation of the problems stated by Fabricant was brought to light when several investigators began to discover strain differences in PPLO. In 1957, Adler and Yamamoto while working with the agent of infectious sinusitis of turkeys found two different strains of PPLO. They found one PPLO strain which was pathogenic and another which was not. These authors in attempting to determine whether PPLO are the cause of infectious sinusitis, cultured PPLO from the exudates of infected poults and used the PPLO from these cultures to

innoculate seven healthy poults. Four days later, four of the seven had sinusitis, (Adler and Yamamoto, 1957).

Later, again in 1957, Adler and Yamamoto report on strain differences in PPLO. Using sterile cotton swabs and selective enrichment media, they were able to culture PPLO from several sites on chickens and turkeys. Also, these authors avoided confusion between PPLO and L-forms of bacteria by culturing the organisms thought to be PPLO in serum which did not contain any antibiotics for ten generations. It should be noted here that the confusion of L-forms for PPLO could possibly be one of the reasons that some investigators are unsuccessful in establishing PPLO as the cause of a disease. Although a large number of PPLO strains were cultured, the authors selected only seven strains for a more intensive study. They were able to demonstrate the presence of at least two different strains of PPLO, (Adler and Yamamoto, 1957).

Yamamoto and Adler report further work along this line in which they studied eight strains of avian PPLO, which they were able to seperate into five groups based on antigenic characteristics, morphology, and physiology. Some of their data are presented in Table V on the following page. (Adler and Yamamoto, 1958). An important aspect of PPLO pathogenicity in animals is the mode of transmission of the agent. Not a great deal is known in this respect. Shirlaw (1949), as already stated in this paper, proposes that the PPLO organism infecting goats may be transmitted through the air. Van Roeckel and others working with chronic respiratory disease in chickens collected data over a period of three years which apparently points to the conclusion that the agent of chronic respiratory disease is transmitted largely through the egg, (Van Roeckel, 1958).

TABLE V

STRAIN DIFFERENCE IN AVIAN PPLO

Strains S6, F, and SU

1. Morphology in broth - coccoid bodies
2. Pathogenicity for birds - positive
3. Tetrazoleum blue reduction - 72 hours
4. Fermentation
   A. Glucose - 32 hours
   B. Sucrose - 240 hours
5. Bile solubility - negative
6. Yeast enhancement of growth on agar - negative
7. Colony size in 48 hours - 0.1 mm

Strain SA

1. Morphology in broth - rings and coccoid bodies
2. Pathogenicity for birds - negative
3. Tetrazoleum blue reduction - 36 hours
4. Fermentation
   A. Glucose - 32 hours
   B. Sucrose - 32 hours
5. Bile solubility - positive
6. Yeast enhancement of growth on agar - marked
7. Colony size in 48 hours - 0.4 mm.

(Yamamoto and Adler, 1958)

In reviewing the problems encountered in studying the pathogenicity of PPLO in animals, it can be seen, that early investigators encountered major difficulties because of one or several of the following reasons. (1) Many workers did not test properly to determine whether they were working with PPLO or the L-forms of bacteria. (2) Because they did not know that strain differences existed between PPLO, they may have sometimes been attempting to induce a disease using a non-pathogenic strain of PPLO. (3) It seems possible that since PPLO are so fastidious, it would have been possible for many investigators in their search for the pathogenic PPLO related to a disease to culture only, or primarily, a non-pathogenic strain, even though the exudates with which they were working did contain pathogenic strains. This could certainly lead to confusion.

Many other problems concerning the pathogenicity of PPLO, avian and otherwise, have been encountered. It seems however, that those discussed here are noteworthy as probably leading to the greatest amount of controversy, confusion, and also, on the positive side of the ledger, to further research.

CHAPTER IV

HUMAN PATHOGENICITY

The problem of the pathogenicity of PPLO in humans is at least as complex as the problem of the pathogenicity of these organisms in animals. The problems in humans are compounded further by the nature of the host. Since the occurence of PPLO in a human was first reported by Dienes and Edsall in 1937, a great deal of research has been done concerning possibilities of human pathogenicity of PPLO, (Dienes and Edsall, 1937). Several important questions immediately come to mind when considering this problem. Are PPLO the cause of any human diseases, and if so, which diseases? Second, how common an occurence is a PPLO infection in humans? How are PPLO transmitted from one human being to another? Is there any way to combat or cure an infection caused by PPLO? Although precise and definite answers are not available to all of these questions, a certain amount is known concerning each. The brief review of the literature in this area, while only barely scratching the surface, should provide a basic insight into the problem.

In 1945, Klieneberger-Nobel reports that she was able to culture PPLO from vaginal smears from 40% of 45 patients attending a venereal disease clinic. From 36 patients attending a gynaecological clinic, PPLO were cultured from 33%. Smears which were positive for PPLO were obtained from only 14% of 50 women patients at an antenatal clinic. Also, she found a particularly high incidence of PPLO in cases involving

an offensive discharge, (Klieneberger-Nobel, 1945). From these data, it appears that although there is a slight correlation between PPLO in the vagina and venereal disease, no definite conclusion can be drawn. Whether or not the high incidence of PPLO in cases involving an offensive discharge is significant or not, is also not established.

In 1953, Freundt presents a report which may be of significance concerning the occurence of PPLO in the female genito-urinary tract. He collected these data over a period of three years, and collected his samples by using sterile cotton swabs. Concerning the study, he states,

> "The main purpose of the present work was to establish the conditions in which micromyces (PPLO) is found in the female genitals. Does it chiefly or exclusively occur in pathological conditions, or may it also inhabit the mucous membranes of a lower genital tract that is quite normal?"

Swabs, mostly from the lower genito-urinary tract were taken from 541 women. 62.1% of the patients suffering from inflammatory genital disease were positive for PPLO. Positive cultures of PPLO were obtained from only 32.7% of those having a normal or healthy genito-urinary tract. A majority of positive cases were found to involve patients whose vaginal pH ranged from 4.9 to 6.3. The author also states, that

> "A grouping of 157 patients according to the general vaginal flora indicated that the occurence of PPLO was closely associated with a mixed population differing from the normal pure Doderlein flora usually associated with a healthy vagina, (Freundt, 1953).

It seems then from this, that although PPLO may be found in a healthy vagina, they are more often found associated with a vagina that is not ideally healthy.

Thus far, we have only dealt with the occurence of PPLO in man. What indications are there that PPLO are directly responsible for human disease, and what can be done to affect a cure? In 1955, Stokes and Lond

reported four cases of human infection with PPLO which appeared to indicate that a PPLO was the causative agent. Three of these cases also seemed to respond to treatment with aureomycin.

In one case, a married woman was admitted who was suffering from abdominal pains and a high fever. At the time of admittance, she was being studied in an attempt to determine whether or not she was sterile. Upon admittance, a vaginal swab was taken which yielded numbers of pus cells and mixed organisms. Upon culture, this first swab showed a heavy growth of anaerobic Streptococci.

The woman was treated for two days with penicillin and sulphonamide. The medication did not seem to have any effect, and her temperature continued to be high. On the third day a second swab was taken, and the patient was treated with streptomycin. The vaginal swab again showed pus cells, but no microbes. Culture of this second swab gave a heavy growth of PPLO. A slight growth of Eschrichia coli was also found. No change in the patient's condition was noted due to the treatment with the streptomycin. Treatment with aureomycin was begun on the fourth day. The patient began to show general improvement, and her temperature was never again over 101 degrees. Later, several abcesses were removed. Upon culture, these abcesses yielded a heavy growth of PPLO. After this minor surgery, the patient had an uneventful recovery, (Stokes and Lond, 1955).

From this case and the others cited by the authors, it would appear that PPLO could be the pathogenic agent involved in at least some human diseases. The possible use of aureomycin as an effective agent against certain PPLO is also indicated. It should be noted however, that the authors do not report any check to determine whether or not what they

called PPLO were actually PPLO or L-forms of bacteria which might possibly have been formed due to the use of the antibiotics.

In 1957, Peoples, Morton, and Feo report that studies of specimens from 34 men complaining of chronic urethritis showed no correlation between the disease and the PPLO found. These authors attempted to culture the PPLO on ordinary Difco PPLO medium, but were unsuccessful. In taking their samples, these authors first cleansed the penis thoroughly with 70% alcohol and let it dry. Next a drop of discharge was collected on two sterile cotton swabs, one of which was observed immediately under a microscope, the other being used to inoculate PPLO broth (Difco), (Peoples, Morton, and Feo, 1957).

In 1960, Shepard reports that in studies made in his laboratory of nongonococcal urethritis, that 70% of over 500 cases studied were associated with PPLO which were of one morphological type. He chose to designate this strain of PPLO as the "T" strain, because of the tiny size of their colonies. The procedures used in the taking of samples by Shepard differs from that used by Peoples et al, (1957). In Shepard's procedure, urethral exudate for primary culture was obtained by deep intraurethral scrapings of the urethral ceiling, employing a 22 gauge platinum inoculating loop bent at about a ten degree angle, (Shepard, 1960).

It is possible that the different results of these last two studies were due to a difference in sampling technique. If Shepard's data are valid, and they seem to be, then it would appear likely that PPLO are involved in at least one disease of the lower genito-urinary tract.

Studies made by Kuzell and Mankle (1960), seem to make a strong case for the involvement of PPLO in human disease and also adds to what little is known of the possible means of transmission of PPLO. It is

significant also, in several cases cited by these authors that apparent cure was achieved by the administering of aureomycin.

In one case cited, a laboratory technician working with PPLO specimens from a patient, splashed some of the PPLO on his hands. He very shortly developed conjunctivitis. He was treated with aureomycin, and an apparent cure was affected. This same technician again accidently spilled the PPLO he was working with on his hands, and again developed the symptoms of conjunctivitis. He was again treated successfully with aureomycin.

Another case is cited by these authors describing the treatment of a woman with a PPLO infection who was successfully treated with aureomycin. It is of interest, that this woman complained of a foul vaginal discharge. Could this be related to the findings of Klieneberger-Nobel?

These data seem to indicate then, that PPLO are definitely involved as the causative agent or are closely linked to the causative agent of several diseases of the lower genito-urinary tract. These diseases might be spread by direct personal contact, and aureomycin seems to be an effective treatment in some cases.

Thus far, only PPLO found in connection with the genitals have been considered. Have PPLO been cultured from other parts of the human body? In 1962, Shklair, Mazzarella, Gutekunst, and Kiggins report the isolation of PPLO from the oral cavities of naval recruits in 87.2% of 211 cases using anaerobic conditions of culture. Under aerobic conditions PPLO were isolated in 75% of 104 cases. PPLO cultured under aerobic conditions formed atypical colonies lacking the usual "fried egg" appearance generally associated with PPLO colonies. Attempts to subculture these atypical colonies under aerobic and anaerobic conditions were

unsuccessful. Typical colonies were formed by the PPLO originally cultured anaerobically. If the typical PPLO isolates were subcultured aerobically, they developed into atypical colonies which as in the first instance, could not be subcultured, (Shklair et al., 1962).

Besides the diseases already mentioned in association with PPLO, recent reports have also indicated a possible connection between PPLO and leukemia, (Cohen, 1965), and a PPLO has been identified as the causal factor of primary atypical pneumonia in man, (Hamilton, 1965).

At present then, it seems that PPLO are associated with several venereal abnormalities, and are definitely the cause of at least one disease, primary atypical pneumonia. Reports indicate that human PPLO are spread through personal contact. Several cases have been cited in which aureomycin seems to have been affective as a curative agent of PPLO infections of the genitourinary tract. Several problems remain. Standard methods of sampling and culturing human PPLO have not been established. It is still difficult to work with human diseases, and a great deal of confusion still exists between PPLO and the L-forms of bacteria.

CHAPTER V

SUMMARY

Although there is still a great deal of controversy concerning the morphology of PPLO, two theories seem to merit most attention. These are discussed and the reasons for the differences of opinion are stated. In studying the relationship of PPLO to other organisms it seems that PPLO might possibly be related to bacteria, probably through the L-forms of bacteria. PPLO and L-forms are similar, especially their individual and colonial morphology, (Diener, 1960). Suggestions by authorities in the field as to the solution of this problem include the further study of bacterial genetics and better methods of distinguishing these organisms from L-forms, (McKay and Truscott, 1960).

Studies of the pathogenicity of these organisms in animals have disclosed difficulties in culturing and identifying different strains of PPLO, (Adler and Yamamoto, 1957) differentiating between PPLO and L-forms, and in obtaining consistent results when attempting to induce disease by the simple inoculation of PPLO into susceptible animals, (Fabricant, 1960). Despite the difficulties encountered however, PPLO have been shown to be the cause of several diseases of animals, some of which are of economic importance.

PPLO have been shown to be the cause of at least one disease of humans and is strongly implicated in the cause of many more, (Hamilton, 1965). In several reports dealing with the relationship of PPLO to venereal infections, it seems that aureomycin is sometimes an effective

treatment, (Kuzell and Mankle, 1960). PPLO have been cultured from several parts of the human body and seem to be a fairly common occurrence. At least one report, (Freundt, 1953), indicates that although PPLO do occur in the healthy vagina, they are more often found in association with the vagina that is not ideally healthy. As in the other problem areas, confusion has been caused in the study of the human pathogenicity of PPLO when investigators did not determine whether they were dealing with a PPLO or merely an L-form of bacteria which might easily been formed due to the use of antibiotics in their culture media.

Although a great deal of controversy and confusion have arisen in the study of PPLO, it should be noted that this confusion has led to further, better controlled research, which in the future should contribute to man's overall understanding of not only these organisms, but life in general.

Selected Bibliography

Adler, H. E. and R. Yamamoto. 1957. Pathogenic and nonpathogenic pleuropneumonia-like organisms in infectious sinusitis of turkeys. American Journal of Veterinary Research, 18, 655-656.

Adler, H. E., R. Yamamoto, and J. Berg. 1957. Strain differences of pleuropneumonia-like organisms of avian origin. Avian Diseases, I, 19027.

Bridie, J. and A. Donatien. 1925. Le microbe de l'agalaxie contagieuse du moutou et de la chevre. Annals of the Institute of Pasteur, 39:925.

Cohen, Gershon J. 1965. American Society for Microbiology, part I of the Symposium on Frontiers of Microbiology. Science, 19(3660), 928-929.

Cordy, D. R. and H. E. Adler. 1960. Patterns of reaction in infection with a virulent form of PPLO from goats. Annals N.Y.A.S. 79:686-695.

Dienes, L. and J. Edsall. 1937. Observations on the organism of Klieneberger. Proceedings of the society of experimental biology and medicine. 36:740.

Fabricant, Julius. 1960. Problems in the isolation and identification of avian PPLO. Annals of the New York Academy of Sciences. 79: 393-396.

Freundt, E. A. 1953. The occurrence of micromyces (pleuropneumonia-like organisms) in the female genito-urinary tract. Acta Pathologica Et Microbiologica Scandinavica. 32:468-480.

Gray, Peter. 1964. Handbook of basic microtechnique. McGraw-Hill Book Company. p 13.

Gross, W. B. 1956. Eschrichia coli as a complicating factor in chronic respiratory disease of chickens and infectious sinusitis of turkeys. Poultry Science. 35:765-771.

Gross, W. B. 1957. Pathological changes of an Eschrichia coli infection in chickens and turkeys. American Journal of Veterinary Research. 18:724-730.

Hamilton, T. R. 1965. Protoplasts and L-forms. Science. 147(3658), 635-638.

Klieneberger-Nobel, Emmy. 1945. Pleuropneumonia-like organisms in the human vagina. Lancet. 249:46-47.

Klieneberger, E. and D. B. Steaben. 1937. On a pleuropneumonia-like organisms in lung lesions of rats, with notes on the clinical and pathological features of the underlying condition. Journal of Hyiene Camb. 37:143.

Kuzell, William C. and Eldon A. Mankle. 1960. Cultivation of pleuropneumonia-like organisms in Rieter's disease, including one instance of laboratory cross infection. Annals of the New York Academy of Science. 79:650-657.

Lecce, James G. Porcine polyserositis with arthritis: isolation of a fastidious pleuropneumonia-like organism and Hemophilus influenzae suis. Annals of the New York Academy of Science. 79:670-676.

Lederberg, Joshua. 1956. Bacterial protoplasts induced by penicillin. National Academy of Science, proceedings. 42:574-577.

Liebermeister, Kurt. 1960. Morphology of the PPLO and L-form of Proteus. Annals of the New York Academy of Sciences, 79:326-343.

Markham, Floyd S. and Sam C. Wong. 1952. Pleuropneumonia-like organisms in the etiology of turkey sinusitis and chronic respiratory disease of chickens. Poultry Science. 31:902-904.

Martin, H. H. 1964. Composition of the mucopolymer in cell walls of unstable and stable L-forms of Proteus mirabilis. Journal of General Microbiology. 36:441-450.

McKay, K. A. and J. R. Taylor. 1954. The reversion of L type cultures previously described as pleuropneumonia-like organisms and associated with chronic respiratory disease of an organism resembling Hemophilus gallinarum. Canadiam Journal of Comparative Medicine and Veterinary Science. 18:7-12.

McKay, K. A. and R. B. Truscott. 1960. Reversion of avian pleuropneumonia-like organisms to bacteria. Annals of the New York Academy of Sciences. 79:465-480.

Nelson, John B. 1957. The enhancing effect of murine hepatitus virus on the cerebral activity of pleuropneumonia-like organisms in mice. Journal of Experimental Medicine. 106:179-189.

Nocard, E. and Roux, E. R. 1898. Le microbe de la peripneumonie. Annals of the Institute of Pasteur. 12:240.

Pease, Phyllis. 1962. Evidence that streptobacillus moniliformis is an intermediate stage between a corynebacterium and its L-form or derived PPLO. Journal of General Microbiology. 29:91-96.

Peoples, Don M., Harry E. and Louis G. Feo. 1957. Unusual pleuropneumonia-like organisms isolated in a study of Trichomonas vaginalis from cases of chronic urethitis. Journal of Bacteriology. 73:398-401

Sabin, Albert B. 1938. Isolation of a filtrable transmissable agent with "neurolytic" properties from Toxoplasma infected tissues. Science. 88:189-191.

Sabin, Albert B. 1938. Identification of the filtrable transmissable neurolytic agent isolated from toxoplasma-infected tissues as a new pleuropneumonia-like microbe. Science. 88:575-576.

Shepard, Maurice C. 1960. Recovery, propagation, and characteristics of the T-strain PPLO isolated from human cases of nongonococcal urethritis. Annal of the New York Academy of Sciences. 79:397-402.

Shirlaw, J. E. 1949. Studies on contagious pleuropneumonia of the goat in India. Indian Journal of Veterinary Medicine and Animal Husbandry. 19:181-213.

Shklair, I. L., M. A. Mazzarella, R. R. Gutekunst, and E. M. Kiggins. 1962. Isolation and incidence of pleuropneumonia-like organisms from the human oral cavity. Journal of Bacteriology. 83:785-788.

Stokes, Joan E. and M. B. Lond. 1955. Human infection with pleuropneumonia-like organisms. Lancet. 268:276-279.

Van Roeckel, H., Olga M. Olesink, and L. P. Beninato. 1958. Epizootiology of chronic respiratory disease in chickens. American Journal of Veterinary Research. 19:453-463.

Yamamoto, Richard and H. E. Adler. 1958. Characterization of pleuropneumonia-like organisms of avian origin, II. Cultural, Biochemical, Morphological, and Further Serological Studies. Journal of Infectious Disease. 102:243-250.

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