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The Pleuropneumonia Group of Organisms: a Review, together with some New Observations

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INTRODUCTION

The organisms of the pleuropneumonia group are of particular interest because, although they have distinctive properties, they resemble in certain respects other widely different organisms. They can exist as particles *c.* 150 m μ . in diameter, i.e. about the same size as the elementary bodies of vaccinia virus, but are nevertheless capable of multiplication in cell-free media. Their relationship to the bacteria has recently been complicated by the finding that under unfavourable conditions many bacteria undergo variation to a so-called L-phase which may resemble morphologically and culturally an organism of the pleuropneumonia group. Cholesterol, which is an essential growth factor for organisms of the pleuropneumonia group, is not known to be required as a nutrient by any bacteria, but is necessary for the growth of certain protozoa and other micro-organisms.

Some organisms of the pleuropneumonia group cause disease and are of practical interest to the veterinarian. Contagious bovine pleuropneumonia is one of the most serious infections of cattle, and even now in some parts of the world causes losses which are difficult to prevent. Other strains have been incriminated as a cause of disease in man, but for this the evidence is much less definite.

Information about the group as a whole and about the individual species is still very incomplete. Much of the investigation has been too limited in scope. Considerable attention has been paid to the morphology of the organisms, but very much less consideration has been given to their physiology. Since the organisms were found to be filterable through candles which held back bacteria they were originally classed with the viruses; many of the strains isolated later were encountered accidentally during the passage of viruses in laboratory animals, and so it happened that they were at first largely studied by the methods used for viruses. More complete examinations were neglected also because of technical difficulties. However, these difficulties can be overcome and the organisms have shown themselves amenable to study by most of the usual bacteriological techniques. Already physiological studies have yielded interesting results and in this review an attempt will be made to reconsider the pleuropneumonia group of organisms in the light of these findings, in order to encourage more intensive studies. These may reveal information of value in the understanding of bacteria and viruses, since the pleuropneumonia group of organisms share properties with both of these, and may also help in establishing the taxonomic relationships of the pleuropneumonia group.

HISTORICAL OUTLINE

It was recognized by Pasteur that contagious pleuropneumonia of cattle was caused by a specific infective agent which could not be seen under the microscope and did not grow in ordinary nutrient broth. In 1898 Nocard & Roux succeeded in growing the agent in a cell-free medium. Its peculiar pleomorphic morphology was described in 1910 by Bordet and also by Borrel and his colleagues, who named it *Asterococcus mycoides*. It was filterable through a Berkefeld V filter, and Elford (1929), using Gradocol filters, showed that cultures contained particles 125–150 m μ . in size. The organism was therefore usually regarded as a virus, and for nearly 25 years occupied a unique position amongst micro-organisms, because, although it had the dimensions of a virus, it was capable of multiplication in cell-free media.

In 1923 Bridré & Donatien showed that contagious agalactia of sheep was caused by an organism similar in morphology and cultural behaviour; this organism was later named *Anulomyces agalaxiae* (Wroblewski, 1931). The morphology and biochemical properties of these two 'viruses' were investigated by a number of workers (Holmes & Pirie, 1932; Ledingham, 1933; Klieneberger, 1934), but it was not until 1934 that another similar organism was isolated by Shoetensack from dogs suffering from distemper. In the following years similar organisms were isolated from a variety of sources; because they resembled the organism of bovine pleuropneumonia, they were called 'pleuropneumonia-like organisms' (PPLO).

Klieneberger (1935) isolated from cultures of *Streptobacillus moniliformis* a 'pleuropneumonia-like organism' which she called L1. At first she regarded it as a symbiont associated with the bacillus, but later (Klieneberger-Nobel, 1949) she agreed with Dienes (1947a) that it was a variant of the bacillus. Klieneberger and her associates (Klieneberger & Steabben, 1937; Findlay *et al.*,

1938, 1939 *a, b*) also isolated pleuropneumonia-like organisms directly from rats and mice. Strains with different pathogenic and antigenic properties were isolated from mice by Sabin & Johnson (1940 *b*). Meanwhile, Laidlaw & Elford (1936) had isolated from sewage pleuropneumonia-like organisms which differed from those isolated from animals by their ability to grow on simpler media without enrichment with serum, and by their lack of pathogenicity for animals. Seiffert (1937 *a, b*) found similar saprophytic strains in soil and decomposing vegetable matter.

A claim by Swift & Brown (1939) to have isolated pleuropneumonia-like organisms from human cases of rheumatic fever aroused interest, but it was later realized that the organisms had been picked up from mice into which the rheumatic exudates had been injected. Subsequent attempts to isolate the organism directly from cases of rheumatism were negative (Sabin & Johnson, 1940 *a*). Organisms of the group were, however, shown to inhabit the human genital tract (Dienes, 1940).

Progress was therefore rapid; whereas in 1934 only two members of the group were known, by 1941 it was realized that there existed a group of organisms, widely dispersed in nature and consisting of numerous species, some pathogenic, some saprophytic, with unique properties which distinguished them from bacteria, rickettsiae and viruses. The available information was excellently summarized by Sabin (1941 *a*) in a review which should be consulted for details of the earlier work.

GENERAL CHARACTERISTICS

Definition

The criteria used by Sabin (1941 *a*) to decide the admission of an organism into the pleuropneumonia group were: '(1) growth in cell-free culture media with the development of polymorphic structures including, "rings", globules, filaments, and minute filterable elementary bodies, usually 125–250 $m\mu$. in size, which are the minimal reproductive units; (2) the development on suitable solid media of characteristic minute colonies which may be as small as 10–20 μ . and as a rule not larger than 600 μ .'. Dienes & Weinberger (1951) pointed out that this definition could be interpreted to include the L-phase variants of bacteria. Edward (1950 *b*, 1953 *a*) suggested that the L-phase organisms ought to be excluded from the pleuropneumonia group, because there appeared to be differences between them and organisms of the pleuropneumonia group proper in colonial appearances, in growth requirements and possibly even in morphology. The relationship of the L-phase organisms to the pleuropneumonia-like organisms will be discussed later (pp. 32–33), and attention will be drawn to those properties which appear to distinguish an L-phase organism from an organism of the pleuropneumonia group.

The reviewer agrees with Sabin that, although organisms of the pleuropneumonia group share many common properties, admission to the group should depend only on the characteristic morphology and colonial appearance. It would be unwise to be more specific than Sabin in describing the morphology,

for interpretation of the microscopical appearances is still controversial. It is, however, possible to be more definite about colonial appearances which differ little from species to species. After 3 days of incubation colonies are translucent and circular with an entire edge, the surface is smooth and raised, sometimes with a small central elevation or depression. Well-isolated colonies of some species reach a diameter of 1.5 mm., but they usually measure 150–300 μ . and are often smaller. Owing to their small size they are best examined with a dissecting microscope ($\times 10$ magnification) using transmitted light. The surface is seen to be faintly marked and there is a central spot of variable size sometimes brown in colour, caused by down-growth into the medium. The colonies of a few species, including the L3 organism from rats (see p. 50) and the α and β strains from dogs (see pp. 49–50), have slightly different appearances to be described later. Exclusion of L-phase organisms from the pleuropneumonia group has been attempted by this description of colonial appearances, but in the opinion of the reviewer the definition of the pleuropneumonia group should include the statement that any organism known to be the L-phase of a bacterium, must not be included in the group.

Classification and nomenclature

The first organisms of the pleuropneumonia group to be isolated were given names; the organism of bovine pleuropneumonia in fact received at least four different names, each based on a different morphological interpretation of the nature of the organism. Symbols were later used to designate the numerous strains, mostly isolated from rodents and identified by their pathogenic and antigenic properties. Sabin (1941*a*) alone attempted a comprehensive classification in which scientific names were given to all the recognized species. Criticisms were immediately raised against his nomenclature; some of the objections had obvious validity, and Sabin (1941*b*) himself soon suggested a revised nomenclature, but this has not been generally accepted.

Sabin considered that organisms of the pleuropneumonia group differed fundamentally from bacteria, particularly in their method of reproduction. He therefore assigned them to a new class Paramycetes, alongside the Schizomycetes. The whole group was included in one order Anulomycetales divided into two families, one containing species isolated from animals and the other containing the saprophytic strains. The different species were grouped together into genera according to their animal host. Previously, Ledingham (1933) had suggested putting the two species known at that time into a new genus to be set up in the existing family of Actinomycetaceae. A third suggestion was to assign them to a new order Borrelomycetales (Turner, 1935*a*).

Thus the proposals made for classifying the organisms varied from placing them in a new genus of an existing family of bacteria to separating them altogether from bacteria into a new class. Because Dienes & Weinberger (1951) believed that the various strains had been derived in the course of evolution from different bacteria, they suggested that the organisms belonged to a morphological group of miscellaneous origin, somewhat analogous to the Fungi Imperfecti, and should not be assigned to one order or family in a natural

system. Agreement on classification and nomenclature can be reached only after deciding their relationship to bacteria, including the L-phase variants of bacteria. When sufficient evidence is available for a decision to be made the problem should be considered by the International Committee on Bacteriological Nomenclature, so that an agreed system of nomenclature may be set up internationally, thus avoiding the repeated changing of names which has caused confusion in other fields. Meanwhile, it is desirable that newly described species should be given symbols rather than proper names. It will thus be possible to avoid the laws of priority fixing names which may later appear unsuitable. Sabin regarded his nomenclature as provisional and did not wish the names he suggested to be fixed by the laws of priority.

Agreement on nomenclature should not be delayed longer than is necessary because there is an urgent need for a less cumbersome term than 'organism of the pleuropneumonia group'. 'Pleuropneumonia-like organism' is often used, but cannot be translated directly into other languages, its abbreviation 'PPLO' also being only applicable in English. Moreover, the use of this term draws an unnecessary distinction between the organism of bovine pleuropneumonia itself and the other species of the group. The term 'L organism' is sometimes used because Klieneberger named some of her original strains L1, L2, L3 and so on. Although this has the advantage of brevity, it should be avoided as it refers only to a provisional system of labelling of strains by one worker. Its use, moreover, has led to considerable confusion because the L1 organism is now recognized to be a variant of a bacillus (*Streptobacillus moniliformis*), whereas L3, L4 and L5 of Klieneberger were members of the pleuropneumonia group proper, isolated directly from animals. Klieneberger-Nobel (1947) later called pleuropneumonia-like variants of other bacteria 'L1 cultures'. Other workers omitted the 'one' and spoke of 'L-type cultures' and 'L forms' (Dienes, 1948; Tulasne, 1951). The prefix 'L' has thus come to be associated with these variants, and in the opinion of the reviewer it should be restricted to them. There will then be no suggestion that, for instance, the L3 organism or the organism of bovine pleuropneumonia are variants of bacteria. Confusion has also arisen because 'L-form' and 'L-body' have been used to describe morphological changes in bacterial cultures, even when attempts to subculture a pleuropneumonia-like L-phase variant have failed. The changes to 'L-forms' or 'L-bodies' may represent L-phase variation, but on the other hand may be degenerative.

There are two subdivisions of the pleuropneumonia group, one containing strains believed to be saprophytic and capable of growing at 22° and on simple media, and the other containing strains which will not grow at 22° and which require media enriched with serum or ascitic fluid. There are a number of species with different pathogenic and biochemical properties, each distinct antigenically. The organism of bovine pleuropneumonia is the type species; it was called *Asterococcus mycoides* by Borrel and his colleagues (1910), and the rules of priority appear to make the name valid. The choice by Sabin (1941*b*) of *Anulomyces agalaxiae* (the organism of contagious agalactia), as the type species from which to build a system of nomenclature, was perhaps unfor-

fortunate in that its properties have been much less studied. Moreover, the organism of bovine pleuropneumonia, the first to be isolated, has already given its name to the group.

Relationship to the L-phase variants of bacteria

When cultures of many different species of bacteria have been exposed to unfavourable conditions, atypical colonies have appeared, composed of organisms which had lost their usual bacillary form. Subculture from such colonies was sometimes successful and could be maintained in series. These atypical organisms are now believed to be variants of the bacteria or a stage in their life cycle. In morphology and colonial appearance these variants or 'L-phase' organisms closely resemble organisms of the pleuropneumonia group. The L1 organism, the first L-phase variant isolated, was originally regarded by Klieneberger (1935) as a pleuropneumonia-like organism which occurred as a symbiont in cultures of *Strep. moniliformis*. Tulasne (1951) believed that an L-phase organism was indistinguishable from an organism of the pleuropneumonia group. Dienes & Weinberger (1951), in a recent review, emphasized that colonies of certain L-phase organisms may appear identical with those of true pleuropneumonia-like organisms.

The similarities, however, between bacterial L-phase organisms and organisms of the pleuropneumonia group may have been overemphasized. Ørskov (1942) described morphological differences between them; these differences were confirmed by Freundt (1950), who did not consider that the L-phase organisms belonged to the pleuropneumonia group. Three L-phase organisms, studied by the present author, differed in their colonial appearance from organisms of the pleuropneumonia group (Edward, 1950*b*; 1953*a*). The colonies of the L-phase organisms were more opaque and their surfaces were more heavily marked than the colonies of the pleuropneumonia-like organisms and they appeared to combine both pleuropneumonia-like and bacterial characteristics; the bacterial characters were even more marked in cultures in fluid and semi-solid media. The published descriptions suggest that cultures of other L-phase organisms also differed from those of true pleuropneumonia-like organisms. Dienes & Weinberger (1951), although they claimed that a colony of a salmonella L-phase organism might be indistinguishable from that of an organism of the pleuropneumonia group, stated that colonies grew to a diameter of 2 mm. and that sometimes growth 'progresses for several weeks and large opalescent colonies develop comparable in abundance to bacterial colonies'. Such colonies could be distinguished from those of true pleuropneumonia-like colonies.

In the opinion of the reviewer a culture can be identified as an L-phase organism when it is examined both with the naked eye and with low magnification at all stages of growth; further assistance is provided by cultures in fluid and semi-solid media. When repeatedly subcultured in penicillin-free media most L-phase organisms tend to revert to the bacillary phase, especially in fluid and semi-solid media. The first few subcultures of an L-phase organism are difficult and often unsuccessful, whereas those of an organism of the

pleuropneumonia group are usually easy on a suitable medium. Klieneberger-Nobel* (1954) also draws attention to these differences between L-phase variants of bacteria and organisms of the pleuropneumonia group proper, and claims that they can be distinguished from each other.

The demonstration that bacteria can exist in an L-phase led to speculation about the nature of those pleuropneumonia-like organisms which exist independently of bacteria. Tulasne (1951) believed that organisms of the pleuropneumonia group were 'des formes L des bactéries fixées et adaptées depuis plus ou moins longtemps à un organisme vivant'. Although Dienes & Weinberger (1951) admitted the existence of an independent group of pleuropneumonia-like organisms, they suggested that the organisms had evolved in the past from bacteria by variation to an L-phase which had become stable and persistent, this change representing a simplification of structure.

An apparent similarity in growth requirements seemed at one time to support the notion that organisms of the pleuropneumonia group and L-phase organisms were closely related. All except the saprophytic strains of the pleuropneumonia group need media enriched with serum or ascitic fluid and so do the L-phase variants of Gram-negative bacteria. Subsequent investigation, however, has indicated an important difference in growth requirements between the two groups of organisms. Cholesterol, or certain other sterols, appears to be necessary for the growth of organisms of the pleuropneumonia group, but not for the growth of L-phase organisms (Edward & Fitzgerald, 1951*b*; Edward, 1953*a*). Two L-phase organisms grew on a medium in which serum had been replaced by a serum albumin fraction + an acetone-insoluble lipid fraction of egg yolk, whereas the further addition of a cholesterol suspension to the medium was required to permit the growth of typical organisms of the pleuropneumonia group. If these findings are confirmed by observations on other L-phase organisms, they suggest a fundamental difference between the two groups of organisms, and it would appear wise to be cautious in speculations upon the origin of the pleuropneumonia group.

Some organisms of the pleuropneumonia group ferment carbohydrates; others appear to lack this power. All the strains with fermentative capabilities tend to form acid from the same carbohydrates; all ferment glucose, maltose, dextrin, starch and glycogen, and some strains also ferment fructose, galactose and mannose (Table 1). No strain was found capable of fermenting the other carbohydrates tested, including lactose, saccharose, mannitol and dulcitol (Edward, 1950*b*). This similarity in fermentative power suggests that the various organisms of the group are closely related to each other. If each species had been derived from a different bacterium and was a stable and persistent form of L-phase variant, as Dienes & Weinberger suggested, the species might be expected to differ from each other in fermentative capabilities, because a bacterium in the L-phase has been observed to ferment the same carbohydrates as it did in the bacillary phase.†

* I am indebted to Dr Klieneberger-Nobel for letting me see her review before it was published.

† See Addendum (p. 64).

Technical methods

Organisms of the pleuropneumonia group are delicate, their nutritional requirements are exacting and their growth is comparatively slow and sparse. These factors make investigations technically difficult, but the difficulties can largely be overcome and the organisms examined by most of the usual bacteriological and serological methods, with certain modifications in technique, provided that adequate amounts of media are available. In order to encourage the complete examination of strains which may be isolated in future a brief description of suitable techniques will be given.

A satisfactory culture medium is clearly essential; its composition will be discussed later. For primary isolation, material (e.g. swabs from the vagina) can be sown directly on solid media; there appears to be no advantage in preliminary cultivation in a fluid medium. Cultivation in a semi-solid medium (Beveridge, 1943) is useful, especially when the organisms in the inoculum are few or are poorly adapted to the medium, but fluid media are not to be recommended except to obtain suspensions. The plates must be incubated in a moist atmosphere, which can be obtained by placing them in a closed vessel containing an exposed piece of moist cotton-wool. Most strains grow satisfactorily under aerobic conditions, but some require anaerobic (or microaerophilic) conditions or an atmosphere of air + 10% CO₂ (Dienes, Ropes, Smith, Madoff & Bauer, 1948; Ruiter & Wentholt, 1952).

In the past, contamination of cultures during prolonged incubation in a moist atmosphere was a frequent difficulty, but this has been overcome by incorporating bacteriostatic substances in the media. A selective medium containing thallium acetate and penicillin permitted the isolation of pleuropneumonia-like organisms in pure culture from highly contaminated material (Edward, 1947*b*). Smith, Morton & Leberman (1950), wishing to avoid penicillin, were successful with a mixture of crystal violet and potassium tellurite. White (1952) used a mixture of thallium acetate, sulphamezathine and crystal violet.

Colonies appear on solid medium in 2–4 days, but plates should be kept 6 days before being discarded as negative. The colonies, although usually visible to the naked eye, are best identified by their characteristic appearance, by means of a dissecting microscope ($\times 10$ magnification) and slightly oblique transmitted light. Examination of impression films stained by Giemsa's method supplies confirmation (Klieneberger-Nobel, 1954). In the opinion of the reviewer examination with a dissecting microscope provides not only the easiest but the most reliable method of diagnosis. It is not an advantage to obtain greater magnification with the two-thirds objective of a microscope. The dissecting microscope, which has a greater depth of focus, gives a more composite picture of a colony. When impression films only are relied on, degenerative or L-phase changes in bacterial colonies may lead to mistakes. Bacteria are particularly liable to form L-phase colonies when penicillin is present in the medium (Dienes, 1947*b*). In preparations examined by dark-ground microscopy pleuropneumonia-like organisms are mimicked by a number of objects, including red cell envelopes and lipid globules.

Subculture should be made from a single colony by means of a Pasteur pipette drawn to a fine capillary point. Subsequent subcultures may be successful with an ordinary loop, but it is preferable to cut out a piece of agar culture, invert it and streak it over the medium to be inoculated. It should be remembered that some species, including the human genital strains, are easier to handle than others and that techniques which succeed with the easier strains may prove unreliable with the more difficult ones. Many strains are delicate and die when cultures are kept for more than a day or two on the bench or at 4°. The only safe way of maintaining them on solid media is by subculture twice weekly. As this is laborious they are best preserved for long periods by drying from the frozen state or by storage at -20°.

Fermentation of carbohydrates has been demonstrated by inoculating serum agar plates containing 1 % carbohydrate and 0.005 % phenol red. To detect haemolysis the organism was grown for 2 days on a serum agar plate; then a thin layer of the same medium containing 5 % of a horse red blood cell suspension was poured over the surface and incubation was continued for a further 2 days. Alternatively, the organism was seeded into serum broth and at daily intervals afterwards 1 ml. of culture was removed. To this sample two drops of a 5 % suspension of horse red cells was added and the mixture was incubated at 37° for 5 hr., with shaking at half-hourly intervals. Changes in colour of the erythrocytes were noted (Edward, 1950*b*).

Suspensions of organisms suitable for serological and other investigations may be obtained by centrifuging broth cultures in the angle head for 1 hr. at 4000 r.p.m. In a fluid culture the maximum number of *viable* organisms is present just as opalescence first appears, or even before. As the culture becomes more opalescent on longer incubation, the viable elements decrease in number although the mass of antigenic material may increase for a time. A considerable economy in medium has been effected by growing the organisms in a layer of fluid on the surface of a solid medium. Serum agar was filled into 4 oz. flat screw-capped bottles, laid on their broad sides to provide a large surface. They were inoculated by pipetting on to the surface a suspension of organisms obtained by washing-off a 2- to 3-day growth on an ordinary plate with *c.* 2 ml. of nutrient broth. The bottles were incubated on their sides and gently rocked once each day. The fluid was finally pipetted off, the organisms in it being concentrated further by centrifugation when required (Edward, unpublished observations).

Nutritional and growth requirements

The first necessity for cultivation *in vitro* is a rich basal medium; most workers have used an ox-heart infusion broth with the addition of 1 % peptone. Some commercially available peptones were more satisfactory than others (Morton, Smith & Leberman, 1951). The addition of a red blood-cell suspension has been recommended, the medium being subsequently boiled and clarified by sedimentation (Klieneberger, 1936; Dienes, 1939). Beveridge (1943) added liver extract. Edward (1947*b*) noticed that growth on media enriched with horse serum varied according to the batch of serum used. When growth was

poor, it was improved by adding a filtrate of a staphylococcal culture or a freshly prepared yeast extract. The growth factor in yeast was not coenzymes I or II (adenine nicotinamide dinucleotides; DPN and TPN). Two old laboratory strains were found to have become sensitive and were inhibited by high concentrations of yeast extract (Edward, 1950*b*). Glucose has been used to improve growth (Sabin, 1941*a*).

The minimum concentration of agar should be employed, since the growth of some strains is impaired by too stiff a gel. The different gelling strengths of different batches of agar cause difficulties. Although some strains grow over a fairly wide range of pH values, others grow best in an alkaline medium, and it is usual to adjust the final pH of the medium to a value between 7.6 and 8.0 (Edward, 1950*b*; Morton, Smith, Williams & Eickenberg, 1951). A few strains, which grew well anaerobically on media at pH 7.0 to 8.0, failed to grow aerobically on a medium at pH 8.0, but grew well aerobically on a medium at pH 6.5 (Edward, unpublished observations).

The nutritional requirements of the pleuropneumonia-like organisms are of special interest as they are the smallest organisms capable of growth in cell-free media. The requirements of the saprophytic strains, isolated from sewage (Laidlaw & Elford, 1936), are simpler than those of the rest of the group. These sewage strains grew in Hartley's broth without the addition of serum; Fildes's peptic digest of red cells improved growth and was essential for the growth of one strain (Pirie, 1937). All other organisms in the group require media enriched with materials such as serum or ascitic fluid; 20% (v/v) of serum or 30% (v/v) ascitic fluid is usually added, although good growth of well-adapted strains can be obtained with as little as 2.5% serum. The sera from different species of animal are not equally effective; some sera appear to be inhibitory in high concentrations (Smith & Morton, 1951*a*; Freundt, 1952*a*). Horse and human sera are suitable for most strains. Many species of pleuropneumonia-like organisms will not grow on media enriched with rabbit serum, but some strains isolated from human saliva grow better on rabbit serum than on horse serum agar (Edward, unpublished observations).

Identification of the growth factors in serum, to allow the replacement of the serum in the medium by simpler or known materials, would seem to be a necessary preliminary to attempts to find a suitable chemically defined medium. When the lipid was extracted from horse serum, neither the lipid fraction alone nor the lipid-free fraction supported growth, but growth occurred on a medium containing both fractions (Edward & Fitzgerald, 1951*b*; Edward, 1953*a*). Serum could be replaced by adding a suspension of cholesterol + bovine albumin fraction V + an acetone-insoluble lipid fraction (AIL) of egg yolk. A degree of growth, which varied in amount with different strains, was obtained with cholesterol alone, cholesterol + starch, cholesterol + bovine albumin and cholesterol + AIL. There was no growth on media containing starch, bovine albumin or AIL, separately or together, without the addition of cholesterol. Strains differed in the relative proportions of cholesterol and AIL required for optimal growth. Excessive concentrations of one or the other were sometimes inhibitory. Cholesterol and stigmasterol promoted growth as well as chole-

sterol, but the cholesteryl esters of acetic, stearic and oleic acids were not effective.

These findings, which suggest cholesterol or another sterol or related compound to be essential for growth, must be reconciled with the findings of Priestley & White (1952) that the serum factor was heat stable and could be separated from the coaguable proteins, and with the claims of Smith & Morton (1951*a*, 1952) to have identified the serum growth factor as a protein of low molecular weight. The examination of a serum fraction, prepared according to Smith & Morton's methods and available commercially, however, showed it to contain lipid, including cholesterol (Edward, 1953*a*). After extraction of the lipid, this serum fraction no longer promoted growth. It thus seems likely that the preparations believed by Smith & Morton to consist only of protein owed their activity to the fact that they also contained lipid which included cholesterol. Haemoglobin solutions, which have been used to replace serum, probably contained lipids derived from the red cell stroma (Gilmore & Sprince, 1949; Smith & Morton, 1951*a*).

The action of serum in promoting growth thus appears to be complex, both protein and lipid fractions being required. The differences in the relative concentrations of cholesterol, phospholipid and protein in the sera of different species of animal probably explain why the sera differ in their ability to promote growth. Rabbit serum usually contains relatively little cholesterol. Strains which failed to grow on a rabbit serum medium grew on a medium containing rabbit serum + cholesterol. Protein and starch may act by binding fatty acid which would otherwise combine with the cholesterol. Oleic acid was shown to inhibit growth (Edward & Fitzgerald, 1951*b*). Attempts to identify the factor in AIL which improved growth were unsuccessful. Cholesterol is required by certain protozoa, e.g. *Trichomonas columbae* (Cailleau, 1937) and by a myxothallophyte *Labyrinthula vitellina* var. *pacifica* (Vishniac & Watson, 1953), but is not known to be an essential nutrient for any species of bacteria. Its requirement by organisms of the pleuropneumonia group is therefore interesting.

Some organisms of the pleuropneumonia group are unable to grow, at least in primary culture, unless another growth factor besides cholesterol is supplied. There was no growth when samples of bull semen were inoculated on the ordinary serum agar medium which was satisfactory for growing other pleuropneumonia-like organisms; positive cultures, however, were obtained from the semen after adding hog gastric mucin to the medium (Edward & Fitzgerald, 1952). Investigations in progress (Edward) suggest that the factor in mucin is deoxyribonucleic acid (DNA). These organisms from the bovine genital tract appear to be defective in their ability to synthesize DNA; in the animal body they probably depended upon the synthetic mechanisms of the host. They thus show a certain similarity to viruses which depend so extensively on the synthetic mechanisms of the host. It is not yet known at what stage in the synthesis of DNA the defect lies. The deoxyribosides of thymine and guanine did not promote growth, so the organism may need to be supplied with a more complex molecule or at least one containing phosphorus and deoxyribose.

Growth of certain other strains of pleuropneumonia-like organisms, in addition to the bovine genital strains, has been found to be improved at first isolation by DNA.

Since strains differ in their optimal growth requirements it is essential that nutritional studies should be carried out with a number of representative strains. It is possible that other organisms of the group exist which have not so far been isolated, because they require other unknown growth factors not present in the media in use or because the proportions of lipid and protein in the sera used for enrichment have been unsuitable.

Growth in the embryonated egg and in tissue culture

Growth has been obtained on the chorio-allantoic membrane of the living chick embryo. Some strains grew poorly and there was better growth after the embryo had been killed by chilling (Sullivan & Dienes, 1939; Swift, 1941). No characteristic lesions were produced, but *Asterococcus mycoides* sometimes caused death of the embryo (Tang, Wei & Edgar, 1936). The organisms were also grown in tissue culture (Sullivan & Dienes, 1939). Recently a strain of *A. mycoides* was found to have become less virulent after serial passage in the embryonated egg, while retaining its ability to immunize (Sheriff & Piercy, 1952). Vaccines prepared in eggs from this strain are being successfully used in the prevention of contagious bovine pleuropneumonia. *Anulomyces agalaxiae* has also been grown on the chorio-allantoic membrane and suspensions of the infected embryonic tissues have been used to prepare vaccines (Lopez & Lopez, 1952).

Morphology

The pleuropneumonia-like organisms are Gram-negative, but are not easily seen in films stained by Gram's method. Numerous techniques which use stained or unstained preparations have therefore been devised for their study. The fact that different workers have used different techniques possibly explains why they have described and interpreted the morphology so differently.

Cultures contain small particles, larger round bodies of various sizes, and filaments. The particles are the minimal reproductive units and can grow into the other forms. One, two or three filaments appear to grow out from a particle; they lengthen and later segment or break up into numbers of small particles, similar to the original particle. This was the only method of reproduction observed by Freundt (1952*a, b*), who regarded a mycelium, exhibiting true branching, as the important morphological feature, the large round bodies being involutionary and of poor viability. Freundt's work is of special interest as he combined experiment with morphological observation; his observations on the effects of nutrition on morphology need extending. The breaking up of filaments into particles, described by Freundt (1952*a*) and illustrated in his micrographs, resembles in many respects the breaking up of filaments, which had been extruded from the cytoplasm of infected cells, to form the elementary bodies of influenza virus (Wyckoff, 1953). Pulvertaft (1953), who made cinematographic records of growth, noted that the small particles appeared sud-

denly, apparently from empty space, as far as microscopical resolution was concerned; they were not formed by division of other particles.

Dienes (1945) drew attention to short rod-shaped forms, often showing bipolar staining, which were formed by segmentation of the filaments, and emphasized their likeness to rod-shaped bacteria. Bacillary forms were observed in electron micrographs; some had a cell wall (Weiss, 1944; Smith, Hillier & Mudd, 1948). Most forms of the organism, however, had no rigid cell wall and were therefore very plastic. Klieneberger & Smiles (1942) believed that the filaments were largely artefacts due to distortion of plastic organisms. They regarded the large round bodies as the important morphological feature. Dienes agreed with these workers that in addition to reproduction by binary fission, these round bodies provided another method of reproduction. Large numbers of particles appeared to develop within the cyst-like round body and were liberated after rupture of the membrane. Segmentation of some round bodies and the extrusion of filaments from others were observed in electron micrographs (Smith *et al.* 1948). This method of development of particles within a large body shows certain similarities to the intracellular multiplication of psittacosis virus. It may be called multipolar germination. Morphological differences have been noted between different species (Sabin, 1941*a*), but, since observations have usually been limited to single strains of a particular species, it is doubtful whether the appearances are sufficiently constant to distinguish the species. Andrewes & Welch (1946) observed a peculiar form of motility, apparently independent of the action of flagella, with certain mouse strains.

Less is known about the appearance of the organisms in the tissues of infected animals. The organisms are too small and pleomorphic to be easily distinguished from other types of granule, but forms similar to those seen in cultures have been observed in exudates (Turner, 1935*b*; Tang, Wei & Edgar, 1936); the organisms were there extracellular. It is less well established whether they also occur intracellularly. Intracytoplasmic granules in the mesothelial cells of the peritoneum of infected mice were considered by Sabin (1941*a*) to be the infecting organisms, but in other infections no cell inclusions were seen (Tang *et al.* 1936). Bacillary and coccal bodies were seen inside pus cells in an abscess produced in a mouse by a strain of human origin (Ruiter & Wentholt, 1952). Intracytoplasmic 'cocco-bacilliform bodies' were found in purulent exudates of mice suffering from infectious catarrh. Pleuropneumonia-like organisms were isolated from the latter lesions, and even after five subcultures they reproduced the disease. Cocco-bacilliform bodies were found in the pus cells of lesions produced by the cultures and they were therefore believed to represent the infecting organisms (Edward, 1947*a*). Similar cocco-bacilliform bodies have been found associated with infections of the upper respiratory tract of fowls and rats, but their relationship to pleuropneumonia-like organisms has not been determined (Nelson, 1935, 1940). Cell inclusions in smears from the human genital tract were described by Harkness (1950), who believed them to be infecting pleuropneumonia-like organisms, but no proof of this was offered.

The recent findings of Klieneberger-Nobel (1954) are of interest. Stained preparations of cultures of material from mice infected with pleuropneumonia-like organisms were made after a few hours of incubation and were examined to find out in which cells pleuropneumonia-like organisms were beginning to multiply to form colonies. Pleuropneumonia-like organisms were demonstrated in leucocytes and other phagocytic cells, but not in fixed tissue cells. Klieneberger-Nobel also showed that stainable bodies which could be mistaken for pleuropneumonia-like organisms appeared in pus cells as a result of degeneration.

Biochemical properties

Some species have biochemical activities which assist their identification (Edward, 1950*b*). Fermentation of carbohydrates with production of acid was demonstrated on solid media by a change in colour of phenol red; the results were somewhat different from those obtained with fluid cultures (Laidlaw & Elford, 1936; Warren, 1942). Some species haemolyse or discolour horse red blood cells (Warren, 1942; Edward, 1950*b*).

Cultures of certain species produce a peculiar 'pearly' film, together with small spots in the medium (Edward, 1950*a*). The spots consist of calcium and magnesium soaps; the composition of the film is more complex, but cholesterol and phospholipid have been detected in it. The formation of a film and spots were best noted on horse serum agar incubated for 6 days. On media enriched with egg yolk spots were formed by some strains which did not form them with horse serum. A clearing of the egg-yolk medium was produced, due to liberation of fatty acids (Edward, unpublished observations). These observations emphasize the importance of lipids in the metabolism of the pleuropneumonia-like organisms. The film and spots are possibly formed by the activity of lipases which break down phospholipid. Pirie & Holmes (1933) showed that the lipid phosphorus was decreased in the medium during growth of the organism of agalactia. The composition of the medium, particularly the proportions of protein and lipid and the amount of fatty acid already present, also determine whether a film and spots are developed on a particular medium by a particular strain.

Holmes & Pirie (1932) studied the utilization of glucose by *Asterococcus mycoides* and showed that this organism and the organism of agalactia reduced methylene blue; lactic acid was the only hydrogen donor identified (Pirie & Holmes, 1933). They subsequently examined the metabolic activities of two of the sewage organisms of Laidlaw & Elford (Holmes, 1937; Pirie, 1937, 1938). Unfortunately there has been no subsequent attempt to extend these observations. Further study of the enzyme systems possessed by these organisms is required, using techniques such as those described by Clarke & Cowan (1952), as this may not only reveal information of fundamental interest, but may also lead to the development of more biochemical tests for identifying species. The organism causing contagious pleuropneumonia of goats is particularly suitable for such studies, as it grows more vigorously than other strains. The observation made by Longley (1951) that this organism liquefied coagulated serum suggests that investigation into the proteolytic activities of the pleuropneu-

monia-like organisms should be made. Holmes & Pirie (1932) detected only a small increase in ammonia in the medium during growth of *A. mycoides* and the non-protein nitrogen, amino nitrogen and ammonia were not appreciably affected by growth of *Anulomyces agalactia* (Pirie & Holmes, 1933).

Organisms of the pleuropneumonia group, in common with the bacterial L-phase variants, possess two other properties which differentiate them from bacteria in the bacillary phase and probably reflect fundamental differences in their metabolism. One is a more or less complete resistance to the bacteriostatic action of penicillin without inactivation of the penicillin, e.g. by a penicillinase. A clue to the mode of action of penicillin might be afforded by determining how pleuropneumonia-like organisms differ from bacteria in their enzyme systems. The other property, which also probably reflects a peculiarity of metabolism, is the growth of surface colonies into the agar medium.

Other properties

Filterability. All ultra-filtration analyses which have been carried out on these organisms have shown that most of the organisms are removed by filtration through a Gradocol membrane of A.P.D. 0.8μ , although the small particles pass membranes of successively smaller A.P.D. The size of the minimal reproductive elements was thus estimated; those of *Asterococcus mycoides* and of the sewage organisms measured 125–175 $m\mu$., those of *Anulomyces agalaxiae* 150–225 $m\mu$. (Elford, 1938; Laidlaw & Elford, 1936), and those of an organism causing pneumonia in mice 165–247 $m\mu$. (Edward, 1940). Ultra-filtration analyses of other strains were reported by Findlay *et al.* (1938, 1939*b*) and by Sabin (1941*a*).

Resistance to physical and chemical agents. The organisms are very susceptible to heat. Some strains are killed by heating at 45° for 15 min., but others withstand 15 min. but not 30 min. at 45° and are killed by 15 min. at 55° (Edward, 1940; Sabin, 1941*a*). They resist freezing and have remained viable for at least one year at -20° . Cultures may also be preserved by freeze-drying. *Asterococcus mycoides* was quickly killed by ether and was lysed by bile salts (Tang, Wei, McWhirter & Edgar, 1935). Other organisms of the group were markedly susceptible to the bactericidal action of soaps (Keller, Smith & Morton, 1952). The inhibitory and lethal actions of various other chemicals were studied by Edward (1947*b*) and by Smith *et al.* (1950).

Sensitivity to chemotherapeutic agents. The first successful attempt at chemotherapy was made by Bridré, Donatien & Hilbert (1928) who showed that stovarsol had a preventive and curative action in contagious agalactia of sheep. Later, infection of rats by the L4 organism attracted interest, because it was thought that in spite of differences in pathology the resulting polyarthrititis might provide an experimental approach to the treatment of rheumatoid arthritis in man. More recently, in view of the possibility that pleuropneumonia-like organisms may cause human genital infection, strains isolated from the genital tract have been tested against the new antibiotics.

Infections of rodents were prevented and cured by organic compounds of gold (Findlay *et al.* 1940), although the organisms were not inhibited *in vitro*

(Sabin & Warren, 1940 *a, b*). Pleuropneumonia-like organisms were not affected by sulphonamides or by penicillin (Beveridge, 1943). Arthritis in rats caused by the L4 organism, although unaffected by penicillin treatment (Powell & Rice, 1944) and by cortisone (Kuzell & Mankle, 1950) was cured by streptomycin (Powell, Jamieson & Rice, 1946), by aureomycin (Kuzell, Gardner & Fairley, 1949) and by terramycin (Kuzell & Mankle, 1950). Human genital strains were inhibited *in vitro* by streptomycin, aureomycin, chloramphenicol and terramycin (Hatch, 1949; Leberman, Smith & Morton, 1950, 1952; Melén, 1951) and could no longer be cultivated from patients who had been treated with these antibiotics (Dienes *et al.* 1948; Brown, Wichelhausen, Robinson & Merchant, 1949). The action of the antibiotics was bacteriostatic rather than bactericidal (Robinson, Wichelhausen & Brown, 1952). Strains varied in the degree of sensitivity and drug-resistance developed against streptomycin (Paine, Murray, Perlmutter & Finland, 1950). Streptomycin, which inhibited the organism of bovine pleuropneumonia *in vitro* (Mornet, Balis & Bachirou, 1949), appeared to act beneficially in the treatment of the disease. Novarsenbenzol was also active in controlling the infection in bovine pleuropneumonia and encouraging preliminary results were obtained with *p*-aminobenzene-sulphonyl-(amino-2-methyl-diazine) (Mornet, Orue & Marty, 1951).

Immunological reactions

The repeated inoculation of rabbits with cultures of pleuropneumonia-like organisms induces the formation of antibodies which are capable of neutralizing pathogenic strains and can be demonstrated by agglutinin, precipitin and complement-fixation reactions. An immune rabbit serum specifically inhibited the agglutination of fowl red cells by a strain of pleuropneumonia-like organism isolated from embryonated hen eggs (Herick & Eaton, 1945). Antisera are also capable of inhibiting growth of the homologous organisms. Some antisera, when added to a horse serum agar plate at a dilution of 1/1000, completely inhibited growth (Edward & Fitzgerald, 1954). The highest dilution at which a serum was inhibitory was not always the same as the highest dilution which caused agglutination; one or other dilution might be greater. Inhibition of growth was unaffected by heating the sera to 56° and so was independent of complement. An antiserum only inhibited the homologous strains and those strains which were shown by the agglutination reaction to be serologically related. In fluid cultures organisms were not killed immediately by the addition of antiserum, although growth was prevented; even 24 hr. later living organisms were detected by subculture.

The growth inhibitory action of antibody has a practical use because the antigenicity of a strain can be tested with only a small amount of suspension. It is also of interest as it represents another difference between organisms of the pleuropneumonia group and bacteria; the latter do not appear to be inhibited by homologous antisera in the absence of complement. An antiserum against the L-phase variant of *Proteus vulgaris* inhibited growth of the L-phase but not that of the bacillary phase. Inhibition of growth shows some similarity to neutralization of a virus by antibody; in both cases combination of antigen

with antibody prevents multiplication although it does not cause immediate death.

Klieneberger (1938, 1940) studied the agglutination reactions of a number of strains of pleuropneumonia-like organisms; she showed that strains of the same species shared common antigens and that there were only occasional cross-reactions at low titre between strains of different species. Subsequent work has confirmed that each species is distinct antigenically. When many strains of a particular species of pleuropneumonia-like organisms were examined antigenic differences between them were noted (Edward, 1950*a*; Edward & Fitzgerald, 1951*a*). Frequently a strain was found which was not agglutinated by an antiserum against a second strain and yet both these strains were agglutinated by an antiserum against a third strain. Species-specific antigens were also demonstrated by the complement-fixation test in strains which failed to agglutinate with a particular antiserum. The organisms thus appear to possess a highly complex antigenic constitution and agglutinin-absorption tests have failed to differentiate between group and type-specific antigens. No evidence has been obtained that the antigenic structure is labile and dependent on the conditions of culture.

Animals after recovery from natural and experimental infections are immune to subsequent infection, but antibodies are not always demonstrable in their sera. Agglutinins, precipitins and complement-fixing antibodies appear sufficiently regularly in contagious bovine pleuropneumonia to provide methods for laboratory diagnosis, but neutralizing antibodies were not found in the sera of infected mice and rats (Sabin, 1941*a*). The complement-fixation test has been successful in the diagnosis of contagious agalactia of sheep and goats (Zavagli, 1951). Complement-fixing antibodies were detected in the vaginal mucus of cattle which were harbouring pleuropneumonia-like organisms in the genital tract (Szabó, 1951). Priestley (1952) showed that the serum of cattle which had recovered from contagious pleuropneumonia or had been immunized against the disease contained antibodies capable of killing *Asterococcus mycoides* in the presence of complement *in vitro*, the effect being similar to bacteriolysis of certain bacteria.

Vaccination against bovine pleuropneumonia is effective only when a living vaccine is used. Cultures of *A. mycoides* attenuated by a sufficient number of serial subcultures in artificial media or by cultivation in embryonated eggs are now used as vaccines. A strain which has been attenuated too much will not confer adequate immunity. Protection against agalactia has been claimed by using as a vaccine cultures of *Anulomyces agalactia*, inactivated with formalin and adsorbed on aluminium hydroxide (Lopez & Lopez, 1952). Cultures inactivated by heating also protected mice against polyarthritis (Sabin, 1941*a*).

Pathogenicity

Four species of the pleuropneumonia group are known to produce natural disease; the three which are of veterinary importance cause, respectively, contagious pleuropneumonia of cattle, contagious agalactia of sheep and goats, and contagious pleuropneumonia of goats, all three being diseases with a high

mortality. The fourth, the L4 organism, was isolated from rats with polyarthritis. Cultures are virulent for the corresponding animal host. The subcutaneous inoculation of *Asterococcus mycoides* produces a spreading and usually fatal cellulitis. The typical lung lesions of pleuropneumonia are not produced when animals are infected by the usual inoculation routes. They have, however, occasionally followed intratracheal injection of virulent lymph and were produced with greater regularity by allowing animals to inhale fine sprays or aerosols of cultures (Campbell, 1938). On repeated subcultivation in artificial media strains of *A. mycoides* lost their virulence. Intravenous injection of the L4 organism produced polyarthritis in rats and the virulence of some strains was retained even after years in artificial culture (Parkes, Wrigley & O'Brien, 1951); other strains quickly lost virulence with repeated subculture. The virulence of a culture appeared to be directly proportional to the speed with which it decolorized methylene blue (Warren, 1942). A strain which was unable to localize in the joints still produced local abscesses in rats and mice (Edward, 1950*b*).

Another group of strains are commensals in rodents. Animals carry the organisms for long periods but remain unaffected unless they are subjected to some experimental procedure. Pleuropneumonia-like organisms have even been isolated directly from brains of healthy mice (Sabin, 1941*a*). Cultures of the pleuropneumonia-like organisms isolated from rodents produced lesions after intravenous injection; some strains retained their virulence during more than 100 subcultures in artificial media (Sabin, 1941*a*). Cultures of other strains were only virulent when mixed with adjuvants such as agar or virus-infected brain tissue (Findlay *et al.* 1938, 1939*b*).

The pathogenicity of several species, including those isolated from the human and bovine genital tracts, is still doubtful (Edward, 1952). Since some undoubted pathogens lose virulence rapidly in subculture while other strains need adjuvants to enable them to produce lesions, investigations of pathogenicity are difficult. The L4 organism is known to be a commensal in rats; some unknown factor must determine whether it initiates polyarthritis. The importance of a second factor in initiating infections by organisms of the pleuropneumonia group was emphasized by Klieneberger-Nobel (1954), who studied the growth of pleuropneumonia-like organisms in the peritoneal cavity of mice infected with ectromelia virus. Organisms may be harmless in their normal habitat and yet produce disease when they reach some other part of the body. The retention of virulence during artificial cultivation may depend on the constitution of the medium.

Infectious catarrh of the upper respiratory tract of mice may be another disease caused by organisms of the pleuropneumonia group. It was reproduced by early subcultures of organisms isolated from lesions, but it is possible that another infective agent which had survived in the cultures caused the disease and produced the typical intracellular 'cocco-bacilliform bodies' (Edward, 1947*a*). The pathogenicity of strains isolated from fowl coryza is likewise unknown. Finally, the pleuropneumonia group includes species which have been found only as commensals, unassociated with disease.

Organisms of the pleuropneumonia group are believed to possess a considerable degree of host specificity. Possibly this conclusion has been based on rather scanty evidence because a wide range of animal species has not been used for testing the pathogenicity of many of the organisms. The most complete investigations have been made with *A. mycoides*; in some experiments cultures infected sheep, goats and buffaloes but not a number of other animals. Some of the rodent strains produced lesions after inoculation in rats and mice. Recently there have been reports that lesions were produced in mice with strains isolated from human infections. Mice inoculated intranasally with these organisms developed pneumonia (Grünholz, 1950; Carlson, Spector & Douglas, 1951), but in assessing the significance of this finding it must be remembered that some stocks of mice harbour strains capable of causing pneumonia (Edward, 1940). Strains isolated from cases of gangrenous balanitis produced purulent lesions after injection, together with agar, into the foot pads of mice (Ruiter & Wentholt, 1952).

The different infections by members of the pleuropneumonia group cause different tissue reactions. Bovine pleuropneumonia chiefly affects the connective tissue, causing acute inflammation with a profuse fibrinous exudate. In agalactia the connective tissue is also involved, but the inflammatory reaction is less acute and is followed by proliferation of fibrous tissue; abscesses are sometimes formed. Some of the strains isolated from mice produced proliferative inflammatory reactions after invading and multiplying within mesenchymal cells (Sabin, 1941*a*). The type A strains which multiplied in this way formed a filterable exotoxin which also had a destructive effect on cells of the brain. An exotoxin pathogenic for cotton rats was formed by the pleuropneumonia-like organism isolated by Herick & Eaton (1945) from embryonated eggs. Reticulum-cell hyperplasia and a polymorphonuclear exudate were produced in mice by strains which caused pneumonia (Edward, 1940). Other strains from rats and mice were pyogenic and produced local abscesses. Some members of the group show marked affinities for certain tissues; a tendency of most species to localize in joints has attracted considerable attention, but there is no evidence that rheumatism in man is caused by pleuropneumonia-like organisms (Findlay, 1946).

DETAILS OF INDIVIDUAL SPECIES

Preliminary remarks

Although a number of different species have been described, many were identified by serological and pathogenic properties alone; sometimes only morphology and cultural appearances were studied. These descriptions are not adequate for comparisons with recently isolated strains to be made. Since many of the original strains were lost, while antisera prepared against them are no longer available, re-examination is impossible. Examination of strains representing as many species as could be collected showed that most species could be recognized by characteristic, cultural and biochemical properties (Edward, 1950*b*, and unpublished observations). Numerous freshly isolated

strains of some species were examined, but with other species only one, or a few, laboratory-adapted strains were available for study and thus their properties may not be completely characteristic of the given species.

All pleuropneumonia-like organisms isolated should be identified when possible according to species. To allow comparisons to be made with the recognized species a collection of freeze-dried cultures of representative strains is maintained at the Wellcome Research Laboratories, Beckenham, Kent together with a number of antisera, also preserved by freeze-drying. Contamination of one organism by another is a constant hazard in bacteriology, but with bacteria this is usually quickly and easily recognized. Unfortunately, when strains of pleuropneumonia-like organisms become mixed, in spite of precautions, this may not be readily apparent because both strains may have identical colonial appearances. Thus the importance of knowing those properties which can be used to check the identity of a particular strain needs no emphasis.

The few cultural and biochemical tests which are at present available for studying strains, are as follows:

(i) *Growth in a semi-solid medium.* Some strains grow equally well throughout a semi-solid medium, others which prefer aerobic conditions grow best near the surface, and a few strains grow better near the bottom than near the surface. The growth may be smooth and fluffy, or granular. Sometimes a strain is neither definitely smooth nor definitely granular.

(ii) *Growth on rabbit serum agar.* Some strains grow poorly on a medium enriched with rabbit serum; others grow as well on a rabbit serum medium as they do on a medium containing horse serum.

(iii) *Formation of a film and spots.* These are noted by growing the strain on horse serum agar for 6 days.

(iv) *Fermentation of carbohydrates.* Some strains ferment certain carbohydrates. Lactose, sucrose, mannitol, dulcitol, sorbitol, rhamnose, xylose, trehalose, raffinose, inositol, inulin and salicin were not fermented by any strain tested; dextrin, starch and glycogen were fermented by those strains which fermented glucose.

(v) *Haemolysis.* Strains produce a variable degree of haemolysis of horse blood agar. The filtrates of 6-day broth cultures of a few strains are capable of discolouring suspensions of horse erythrocytes. Table 1 shows how these tests assist the identification of species.

The numerous organisms which have been described can be conveniently grouped according to habitat, but this arrangement is only provisional. Sabin (1941*a, b*) classified and named species according to the animal host, but physiological properties, when more becomes known about them, seem likely to provide a more suitable means of classification, as with bacteria. It is already apparent that there is a difference between those species which ferment carbohydrates and those which do not.

Organisms from cattle

Asterococcus mycoides, the type species, causes contagious pleuropneumonia. It grew poorly on rabbit serum agar and did not form a film and spots on horse

serum agar (Table 1). In a semi-solid medium it grew best near the surface, producing a smooth growth (Edward, 1950*b*). When a broth culture was shaken the sedimented growth rose in characteristic silky swirls (Walker, 1930). It fermented glucose, fructose, maltose, mannose, dextrin, starch and glycogen, but not galactose; slight fermentation of sucrose and trehalose has also been reported (Tang *et al.* 1935). Indole was not formed. The organism produced haemolysis on horse blood agar and differed from all other organisms of the group except the organism causing pleuropneumonia of goats in that discoloration of horse erythrocytes was produced by filtrates of 6-day fluid cultures (Warren, 1942; Edward, 1950*b*). It also liquefied inspissated serum (Edward, unpublished). For reasons stated later (p. 48), it is suggested that this organism should be known as *A. mycoides*.

P strains. Organisms of a different species have been isolated from the bovine genital tract (Edward, Hancock & Hignett, 1947). All strains which required serum for growth produced a film and spots on horse serum agar and were regarded as a single species, provisionally designated by the letter P (Edward, 1950*a, b*). The serological examination of ten strains showed that they shared common antigens. The organisms grew equally well throughout a semi-solid medium, giving a smooth growth (Table 1). They grew only poorly on rabbit serum agar. They produced haemolysis of horse blood agar but did not ferment carbohydrates. It is possible that P strains may cause inflammation of the genital tract, predisposing to infertility, but the experimental inoculation of heifers *in utero* with cultures was without effect (Edward, 1952).

S strains. Occasionally strains capable of growth on serum-free media were isolated from samples taken from the genital tract (Edward, 1950*a*). These S strains were probably not inhabitants of the genital tract but reached samples as contaminants; they are included among the saprophytic strains.

Organisms from sheep and goats

Organism of contagious agalactia of sheep and goats. Although this organism, named *Anulomyces agalaxiae* by Wroblewski (1931), was first isolated in 1923 by Bridré & Donatien, who pointed out its similarity to the organism of contagious bovine pleuropneumonia, there have been few attempts to study its cultural and biochemical properties for comparison with those of the organism of bovine pleuropneumonia. In continental literature it is still the practice to call the organism of agalactia a virus (Zavagli, 1951), and this fact may possibly account for the failure to study its cultural properties adequately. In view of the importance of the organism in veterinary medicine it was therefore necessary for this review to collect strains for study. Five strains were obtained through the kindness of Prof. P. Hauduroy from Dr Anguelov of Sofia, from Dr C. Lopez of Madrid and from Prof. V. Zavagli of Rome. Four of the five strains produced a film and spots on horse serum agar; a film containing lipids on the surface of fluid cultures has been described previously (Pirie & Holmes, 1933; Nowak & Lominski, 1934). All the strains grew best near the surface of a semi-solid medium producing a smooth growth; they only grew poorly on

rabbit serum agar (Table 1). They produced haemolysis on horse blood agar, but did not ferment carbohydrates (Edward, 1953*b*).

In addition to these five strains of *A. agalaxiae* a sixth strain, also believed to be the organism of agalactia, was received. The properties of this strain, however, were different from those of the other six strains and were identical with those of organism of goat pleuropneumonia (see below). This emphasizes the necessity to examine every strain of pleuropneumonia-like organism sufficiently for its species to be identified; the identification of strains from goats is particularly important, because two species of the pleuropneumonia group are pathogenic for this animal.

Organism of contagious pleuropneumonia of goats. It was only recently recognized that contagious pleuropneumonia of goats was caused by an organism of the pleuropneumonia group. This disease has been reported from many parts of Europe, Asia and Africa. It is of considerable economic importance as it is almost always fatal; in Nigeria alone it is estimated to cause losses of £70,000 per annum. The infective agent was believed to be a filterable virus until Longley (1951), whose work was confirmed independently by Beveridge & Chu (personal communication), isolated a pleuropneumonia-like organism and reproduced the disease with cultures. Lesions were produced in sheep by experimental inoculation, but the sheep did not acquire the disease under natural conditions. The organism was serologically distinct from the organism causing bovine pleuropneumonia.

Well-separated colonies of two strains, one isolated by Longley and the other by Chu, reached a diameter of 1.5 mm. after 3 days of incubation and were thus noticeably larger than those of any other organism of the group. Chu's strain grew best near the surface of a semi-solid medium, producing a smooth growth; it grew well on rabbit serum agar and did not form a film and spots (Table 1). There was slight growth on the basal medium alone without serum enrichment. The organism liquefied inspissated serum and fermented glucose, fructose, maltose and mannose, but not galactose. It produced haemolysis of horse blood agar. The supernatant from a 6-day fluid culture discoloured horse erythrocytes and it was the only strain, apart from the organism causing bovine pleuropneumonia, which showed this property (Edward, 1953*b*).

Pleuropneumonia of goats resembles bovine pleuropneumonia in symptomatology and pathology; the causative organisms have similar properties and the only significant differences noted between them were in host and in antigenic constitution. It is possible that the disease in goats is caused by a bovine organism which has become adapted to goats. Under experimental conditions goats were infected with cultures of the bovine organism, grown in media containing sheep serum (Dujardin-Beaumetz, 1906). The susceptibility of cattle to the goat organism does not appear to have been tested experimentally, but cattle do not acquire the disease from infected goats under natural conditions. It is therefore suggested that the organism of goat pleuropneumonia should not be assigned to a new species, but should provisionally be regarded as a new variety, to be called *Asterococcus mycoides* var. *capri*.

Melanidi (1951) drew attention to an infective oedema and cellulitis which was endemic among goats around Sparta in Greece and was usually fatal. The disease was regarded as a hyper-acute type of contagious agalactia, because the causative organism, a member of the pleuropneumonia group, appeared to be serologically the same as the organism of contagious agalactia. It is, however, apparent that this naturally occurring infection produces a condition similar to that produced experimentally by the subcutaneous inoculation of the organism of goat pneumonia. A strain of the organism, isolated from an infected goat in Sparta and kindly provided by Dr Melanidi, had the cultural and biochemical properties of the organism of goat pneumonia and not those of the organism of agalactia (Edward, 1953*b*). Further investigation should therefore be made into the aetiology of the disease in Sparta, and it would seem that the disease is more likely to be caused by the organism responsible for pleuropneumonia in goats than by the organism of agalactia. The value of examining strains of organisms by cultural and biochemical methods, as well as by serological methods, must be emphasized.

Other strains from sheep. Pleuropneumonia-like organisms were isolated from the feet of sheep affected by foot rot (Beveridge, 1941). Cultures of the organisms did not reproduce the disease and properties of the strains were not studied further.

Organisms from dogs

Shoetensack (1934, 1936*a, b*) isolated organisms of the pleuropneumonia group from the tissues and nasal secretions of dogs suffering from distemper. The strains belonged to two types, *Asterococcus canis* types I and II, which differed from each other serologically (Klieneberger, 1938). Their pathogenicity was uncertain. *A. canis* type I was believed by Shoetensack to be the cause of the distemper, *A. canis* type II being possibly a secondary invader. Since secondary infection is an important feature of the disease caused by distemper virus, it is possible that both types of organism were secondary invaders. These organisms of Shoetensack cannot now be compared with others more recently isolated from dogs, because cultures appear to be no longer in existence, and the original descriptions were inadequate.

Edward & Fitzgerald (1951*a*) found pleuropneumonia-like organisms in a high proportion of cultures from throat and vagina of bitches. In some cultures there were three different types of colony and by subcultivating each type separately organisms with different colonial and serological properties were obtained. Most of the strains belonged to three species, provisionally designated as α , β and γ ; a few strains which appeared different were not studied further. None of these species is known to be pathogenic, but the α strains merit further investigation for possible pathogenicity.

The α strains, isolated from vagina and semen, formed coarsely reticulated colonies; the centre appeared to contain large globules which tended to obscure the central spot, while the periphery was transparent. These colonial characteristics were lost on repeated subculture. The organisms grew poorly on rabbit serum agar and did not form a film and spots on horse serum agar (Table 1). They grew throughout semi-solid media, but their growth was neither

definitely granular nor completely smooth. Glucose was not fermented and horse blood agar was only feebly haemolysed.

The β strains, isolated from vagina and throat, formed relatively large flattened colonies with small poorly developed central spots. These characteristic appearances were quickly lost by subcultivation. The strains differed from the α strains by growing well on rabbit serum agar and by giving more marked haemolysis; they were also serologically different (Table 1).

The γ strains, also isolated from vagina and throat, produced a film and spots on horse serum agar; their colonies were not otherwise distinctive. They grew only poorly on rabbit serum agar, produced haemolysis of blood agar and did not ferment glucose (Table 1).

Organisms from rats

L3 organism. Numerous isolations of a pleuropneumonia-like organism, known as the L3 organism, were made by Klieneberger & Steabben (1937, 1940) from the lungs of laboratory rats; most of the animals had bronchiectasis but some were without lung lesions. Once the organism was isolated from a wild rat. Four strains from laboratory rats and the one strain from a wild rat were serologically similar (Klieneberger, 1938). The association of the organisms with bronchiectasis suggested that they were pathogenic, but the inoculation of cultures into rats was without effect, although abscesses were produced locally in mice by injecting cultures subcutaneously together with agar.

The L3 organism differed from other strains in its colonial appearances. The surface of a colony was somewhat rough and the surface markings tended to obscure the central spot. When one of the original strains was re-examined, it produced a film and spots on horse serum agar, grew poorly on rabbit serum and grew better near the surface of a semi-solid medium, the growth being granular (Table 1). It haemolysed horse blood and fermented glucose, maltose, and mannose, but not fructose or galactose (Edward, 1950*b*). Unfortunately this strain was lost. Dr E. Klieneberger-Nobel kindly provided another strain which she had recently isolated from the lung of a rat. The new strain differed from the original in several respects. It did not have the same colonial appearances; it grew equally throughout a semi-solid medium, did not form a film and spots and fermented fructose, feebly, but not mannose. Moreover, it was not pathogenic for mice on subcutaneous inoculation. Possibly more than one species inhabit the lungs of rats. The L3 organism has also been found in mice. Klieneberger & Steabben (1940) isolated one strain from the brain of a mouse and a strain isolated by Edward from the nose of a mouse with infectious catarrh was serologically identical with the L3 organism (Klieneberger & Smiles, 1942). The similarity between the L3 organism and strains isolated from mice with infectious catarrh is discussed on p. 52.

The L4 organism. The antigenically different L4 organism was originally isolated by Klieneberger (1938) from the submaxillary gland of a rat; it was serologically identical with a pyogenic agent which contaminated a transmissible sarcoma (Klieneberger, 1939) and with a pleuropneumonia-like

organism which caused polyarthritis in rats (Findlay *et al.* 1939*b*). It produced abscesses when inoculated into rats and mice; in rats there was a tendency to localize in the joints. Intracerebral injection of mice caused encephalitis.

Pleuropneumonia-like organisms with similar properties were isolated by other workers from wild and laboratory rats with polyarthritis, but their identity with the original L4 strain was not checked serologically (Beeuwkes & Collier, 1942; Preston, 1942). The strain isolated by Preston grew well on rabbit serum agar, did not form a film and spots, gave a smooth growth throughout a semisolid medium and haemolysed blood, but did not ferment carbohydrates (Table 1) (Edward, 1950*b*).

Organisms from mice

Many different strains of pleuropneumonia-like organisms have been isolated from mice, and their relationship to each other is confusing; possibly some are identical with organisms found in the lungs of rats.

Sabin's strains. Sabin (1941*a*) isolated five serologically different types of organism from nose, conjunctival sac, respiratory tract and even brain of normal mice. Sometimes three different types of organism were isolated from the same culture (Sabin & Johnson, 1940*b*). Intravenous inoculation of mice with types, A, B, C, D and E produced arthritis. Type A was also capable of multiplication in the brain and caused encephalitis after intracerebral inoculation. The affected mice exhibited peculiar nervous signs known as 'rolling disease'. Sabin's strains no longer exist for comparison with other strains.

The L5 organism, serologically identical with Sabin's type A, was isolated by Findlay and his colleagues (1938) from the brains of mice used for passaging lymphocytic choriomeningitis virus. It differed from Sabin's strain in being less virulent and in not forming exotoxin. A strain of L5, which had lost its virulence after several years in subculture, grew poorly on rabbit serum agar, did not form a film and spots, haemolysed blood and fermented glucose, maltose and mannose, but not fructose or galactose (Table 1) (Edward, 1950*b*). It gave a granular growth, best near the surface of a semi-solid medium, whereas its growth originally had been smoother (Klieneberger, 1940).

The L6 organism was isolated from the brains of mice which had been inoculated with mouse blood containing *Eperythrozoon coccoides* (Findlay *et al.* 1939*a*). Its colonies, larger than those of L5, were coarsely marked and vacuolated; growth in broth was granular. It differed from the L5 organism serologically (Klieneberger, 1940).

From infectious catarrh. Pleuropneumonia-like organisms were found associated with infectious catarrh, a chronic infection of the respiratory tract of mice, frequently complicated by otitis media and pneumonia. The disease is endemic in some stocks of laboratory mice and intranasal inoculation of infected mice caused pneumonia which was transmissible in series (Edward, 1940). Nelson (1937), who at first failed to isolate pleuropneumonia-like organisms from lesions in this disease, ascribed the infection to 'cocco-bacilli-form bodies', visible inside pus cells. These bodies were grown in tissue culture. Later, he agreed that pleuropneumonia-like organisms were associated with

the disease (Nelson, 1948). Early subcultures of the organisms reproduced the disease and cocco-bacilliform bodies were present in the lesions (Edward, 1947*a*).

Strains isolated from mice with catarrh differed in their colonial appearances. The colonies of some strains had the appearance typical of organisms of this group; the colonies of other strains resembled those of the L3 organism and had a rougher surface. Recently pleuropneumonia-like organisms were isolated on numerous occasions from a strain of infectious catarrh, maintained in mice by serial passage (Edward, unpublished observations). Primary isolation was possible on solid media, although this had not been accomplished in earlier work. Growth in the primary culture was improved by the addition of thymus nucleic acid to the medium. All the strains had the same properties; their colonies were of the rough type. They grew well on rabbit serum agar, formed a film and spots on horse serum agar, haemolysed blood and fermented glucose, maltose and mannose; fermentation of fructose was weak and variable and there was possibly slight fermentation of galactose (Table 1). In a semi-solid medium growth was better near the surface; growth of a freshly isolated strain was smooth, but it became granular during subcultivation. Previously reported findings must be disregarded as the strain examined was later found not to have originated from a mouse (Edward, 1950*b*).

Although the strains lost their ability to produce pneumonia after a few subcultures, they produced abscesses even after many subcultures when inoculated subcutaneously into mice together with agar. Cocco-bacilliform bodies were not visible in the pus from these abscesses. The organisms thus resemble the L3 organism in pathogenic and cultural properties, and it is possible that the same organism inhabits both mice and rats. Possibly organisms of different species were found in cultures from mice belonging to different stocks. An antiserum prepared against one strain failed to agglutinate two of five other strains (Edward, 1940).

From conjunctivitis. Pleuropneumonia-like organisms were found associated with endemic conjunctivitis (Nelson, 1950*a, b*). Their colonies differed from those of strains isolated from mice with catarrh. Cultures did not cause conjunctivitis unless very heavy suspensions were instilled into the eye.

Organisms from fowls

A pneumonia virus which was being passaged in embryonated eggs was found to be contaminated with a pleuropneumonia-like organism. Cultures of the organism produced haemagglutination, which was inhibited by an antiserum prepared in a rabbit and by sera from a high proportion of hens in the hatchery providing the eggs. It thus seemed likely that the strain had been picked up from the eggs (Herick & Eaton, 1945).

An infective agent has been isolated from cases of upper respiratory catarrh in fowls; after passage in embryonated eggs it reproduced the disease in fowls. A similar agent was isolated from turkey sinusitis. Both agents were visible microscopically in infected tissues and were regarded as viruses (Van Roekel, Olesiuk & Peck, 1952). Markham & Wong (1952) isolated pleuropneumonia-

like organisms from egg-passage material containing each of the agents. After thirteen successive subcultures in artificial media the organisms produced specific lesions in chick embryos. Suspensions of the tissues of these embryos produced catarrh in fowls. Infections of the upper respiratory tract of fowls and turkeys are of considerable economic importance, and further work is needed to establish the significance of the pleuropneumonia-like organisms and their relationship to the viruses and cocco-bacilliform bodies which have been found associated with these infections (Nelson, 1935). Pleuropneumonia-like organisms have been isolated from the upper respiratory tract of apparently healthy fowls, and even strains isolated from birds with catarrh have not produced lesions after experimental inoculation (Beveridge & Chu, personal communication).

A strain of pleuropneumonia-like organism isolated from the upper respiratory tract of a fowl was received from Dr H. P. Chu. The organism formed a film and spots on horse serum agar, grew well on rabbit serum agar and grew throughout a semi-solid medium as a smooth growth (Table 1). It haemolysed blood but did not ferment glucose (Edward, unpublished observations).

Organisms from man

Pleuropneumonia-like organisms have been shown to be frequent inhabitants of the human genital tract and anal canal (Dienes, 1940; Dienes *et al.* 1948; Harkness, 1950). They were isolated from the urethra of about 20 % of cases of non-specific urethritis in man. As urethral cultures from healthy males were at first negative, it seemed possible that the organisms were a cause of non-specific urethritis (Beveridge, Campbell & Lind, 1946; Harkness & Henderson-Begg, 1948). However, in two more recent investigations the organisms were isolated nearly as frequently from healthy males as from patients with urethritis (Harkness, 1950; Melén & Linnross, 1952). Even more frequent isolations were made from cervix and vagina of women; the proportion of positive isolations was significantly greater in those with inflammation of the genital organs than in those without inflammatory lesions (Klieneberger-Nobel, 1945; Randall, Stein & Ayres, 1950; Melén & Odeblad, 1951, 1952).

The organisms appear to be transmitted usually by sexual intercourse, but their role in causing inflammation of the genital tract is doubtful (Edward, 1952). A recent investigation tends to confirm the view that the pleuropneumonia-like organisms are not the cause of non-specific urethritis (Nicol & Edward, 1953). This disease in patients with positive cultures for pleuropneumonia-like organisms did not differ in its clinical course or in its response to treatment from the disease in those with negative cultures; there is therefore no evidence for regarding the organisms as important secondary invaders. Although the organisms appear to be commensals, the possibility cannot be excluded that they may cause suppurative lesions under special circumstances.

A search has been made for pleuropneumonia-like organisms in joint fluid from cases of arthritis which had occurred as a complication of non-specific urethritis. Positive cultures were obtained from a few cases, but the cultures were more often negative (Dienes *et al.* 1948; Kuzell & Mankle, 1950). The

significance of the positive findings is doubtful, because the isolations of pleuropneumonia-like organisms from brains of healthy mice suggest that organisms of the pleuropneumonia group are able to remain latent inside the body.

A total of ninety-one strains of pleuropneumonia-like organisms, isolated in London from genital tract and anal canal, had, with one exception, the same biological properties and were serologically related to each other (Nicol & Edward, 1953). Two strains isolated in Paris and two of five strains from the U.S.A. were also similar. These strains will be referred to as human type 1. They grew well on rabbit serum agar and did not form a film and spots (Table 1). Growth, which was usually markedly granular, occurred throughout semi-solid media. They did not ferment glucose and produced little or no haemolysis of horse blood agar. All the strains were related to each other serologically, although they reacted differently with antisera prepared against four of them. One strain, H. 23, was not agglutinated by antisera to three other strains, yet an H. 23 antiserum agglutinated to full titre strain H. 26, which itself was agglutinated by the other three antisera. Strains isolated from persons with no evidence of genital infection did not differ in their serological or other properties from strains isolated from patients with genital infection; strains from rectum and anal canal were similar to those from cervix and urethra.

A few strains were isolated on media incubated anaerobically, the corresponding cultures on media incubated aerobically being negative. The strains grew well on media at pH values between 7.0 and 8.0 when the plates were incubated anaerobically or in an atmosphere containing 10% CO₂, there being little or no growth under aerobic conditions. Good growth, however, was obtained aerobically on media adjusted to pH 6.5 and 6.0. These strains did not differ from the usual type 1 strains in their serological or other properties.

Five strains isolated in the U.S.A. were received from Prof. H. E. Morton; three of them did not react with any of the type 1 antisera, but resembled each other serologically. They also differed from type 1 strains, because abscesses were produced locally when cultures containing agar were injected subcutaneously into mice. Their biochemical properties were the same as those of type 1 (Table 1). They will be provisionally called human type 2. One of the three strains, 'Campo L', was originally isolated by Dr Dienes and found to be serologically similar to five other strains isolated by him in 1939-40 (Dienes, personal communication). Four other strains isolated in America were shown to be identical serologically with 'Campo L' (Norman, Saslaw & Kuhn, 1950).

Pleuropneumonia-like organisms, which appeared to differ from the type usually isolated from the genital tract, were cultured by Ruiter & Wentholt (1952) from four patients with ulcerative lesions of the glans penis; they were called 'G-strains'. They were associated with fusiform bacilli and other bacteria, so that their pathogenic significance was uncertain. They were difficult to grow and needed anaerobic or microaerophilic conditions. Abscesses were produced when cultures were inoculated into the foot pads of mice. A strain which had

lost its virulence for mice was kindly provided by Prof. M. Ruiter. Its growth was improved by adding thymus nucleic acid to the medium, when it became as good as the growth of type 1 strains provided the cultures were incubated anaerobically. Under aerobic conditions there was only a trace of growth on media adjusted to pH 7.5–8.0, but there was moderately good growth on media at pH 6.5. The strain also grew moderately well in an atmosphere containing 10 % CO₂. A film and spots were formed on horse serum agar; growth on rabbit serum agar was as good as on horse serum agar (Table 1). In a semi-solid medium growth which was smooth was found near the bottom of the tube. Glucose, fructose and maltose were fermented, but not mannose or galactose. Only a trace of haemolysis was produced (Edward, unpublished observations). This strain will be designated as human type 3.

All the ninety-one strains isolated in this laboratory from the genital tract were identified as type 1 with the exception of one strain, H. 106. This strain differed serologically from types 1, 2 and 3, but resembled type 3 in fermenting the same carbohydrates and in forming a film and spots (Table 1). It grew well anaerobically without adding thymus nucleic acid to the medium, but under aerobic conditions its growth was negligible.

Pleuropneumonia-like organisms also frequently inhabit the mouth and pharynx. Morton and his colleagues (Morton *et al.* 1951, 1952; Smith & Morton, 1951*b*) isolated strains on media enriched with horse serum incubated aerobically, but in this laboratory successful isolations were only obtained under anaerobic conditions. Growth was better on rabbit serum agar than on horse serum agar and was improved by adding thymus nucleic acid to the medium. When the thymus nucleic acid was not added growth often took place only near bacterial colonies; it is possible that the failure to subculture the 'X' colonies noted by Sabin & Johnson (1940*a*) was due to absence of thymus nucleic acid from the medium.

Three of five strains isolated from saliva formed a film and spots. Although two strains did not exhibit this property, in other respects the five strains behaved similarly. They gave a smooth growth at the bottom of semi-solid media; they did not haemolyse horse blood and did not ferment glucose (Table 1). An antiserum prepared against one strain inhibited the growth of each of the five strains, although it failed to agglutinate the homologous organism to a significant titre. Growth was not inhibited by antisera to the types of organism isolated from the genital tract (Edward, unpublished observations). These strains will be called Human Type 4. Dienes & Madoff (1953) showed that the strains they isolated from the mouth differed serologically and in colonial appearances from the genital strains. Their strains, like those of Morton and his colleagues, were isolated under aerobic conditions without adding thymus nucleic acid to the medium. It is not yet clear what relationship the American strains bear to those isolated in this laboratory, which require different cultural conditions.

Paine and his colleagues (1950) reported the isolation of a pleuropneumonia-like organism from a patient with meningitis and a brain abscess, which had resulted from a tobacco pipe being thrust into the brain through the orbital

wall. A streptococcus was also present in the pus, so that the infection could not with certainty be ascribed to the pleuropneumonia-like organism. The pleuropneumonia-like organism did not grow anaerobically; haemolysis and fermentation of carbohydrates were not detected.

Pleuropneumonia-like organisms were also isolated from the blood of another patient with a brain abscess and from the blood of three children with purpura (Carlson *et al.* 1951). It is interesting that one of the infections followed a human bite. The strains, which are no longer available, were not sufficiently studied for their relationship to the genital and mouth strains to be established. Although they were not proved to be responsible for the infections, these reports suggest the wisdom of looking for organisms of the pleuropneumonia group in infections of unknown aetiology.

Saprophytic strains

These are capable of growing on serum-free media without the addition of cholesterol. The optimum temperature for growth is 30°, but moderate growth occurs at 37 and 22°. They were first isolated from sewage by Laidlaw & Elford (1936), and from decomposing vegetable matter by Seiffert (1937 *a, b*). Similar strains were also encountered as contaminants in cultures made from the genital tract of cattle (Edward, 1950 *a*). The sewage A and B strains of Laidlaw & Elford, although differing somewhat serologically, shared common antigens; Seiffert's strains were closely related to sewage A (Klieneberger, 1940). S strains encountered in cultures from the bovine genital tract were also serologically related to sewage A and B. An antiserum prepared against one S strain fixed complement with suspensions of sewage A and B, although it did not agglutinate them. One of Laidlaw & Elford's strains, sewage C, was antigenically distinct from sewage A and B, and differed in its nutritional requirements by requiring haemin (Pirie, 1937, 1938; Holmes, 1937). It is unfortunate that this strain, which was of a different type from the others, appears to have been lost.

Sewage A and B strains did not form a film and spots; they grew best near the surface of semi-solid media (Table 1). Horse blood was haemolysed and the strains fermented glucose, fructose and maltose, but not galactose or mannose. An S strain gave the same reactions, except that it fermented galactose (Edward, 1950 *b*).

CONCLUSION

The nature of the pleuropneumonia group of organisms must be considered in the light of their properties. They have on the one hand been classed as viruses, and on the other have been included among the bacteria. Like bacteria, they can be grown on cell-free media; most strains appear to need cholesterol or another sterol for growth, whereas cholesterol is not known to be an essential nutrient for any bacteria. Some strains when first isolated from animals appear to be defective in synthesizing deoxyribonucleic acid; bacteria synthesize their own nucleic acids and the most exacting require to be given only certain purines, pyrimidines and nucleosides, but viruses depend in this and other respects on the synthetic mechanisms of the cells they parasitize. The pleuro-

pneumonia-like organisms have distinctive morphological and colonial appearances, but some bacteria form a variant or L-phase which resembles a pleuropneumonia-like organism in morphology and colonial appearance. In addition to division by binary fission the pleuropneumonia-like organisms appear to be capable of multiplication by multipolar germination in a manner somewhat similar to the intracellular multiplication of certain viruses. An understanding of the mode of reproduction of viruses may possibly be obtained by studying the growth and multiplication of organisms of the pleuropneumonia group, because there appear to be certain similarities between the two methods.

Cultures of organisms of the pleuropneumonia group contain elements as small as the particles of some true viruses. Pleuropneumonia-like organisms also exhibit other similarities to viruses. The injection of hyperimmune serum into an animal before inoculating it with a pathogenic member of the pleuropneumonia group will prevent infection. Hyperimmune sera are of little use in preventing bacterial infections, whereas most viruses can be neutralized both *in vitro* and *in vivo*. Growth of a pleuropneumonia-like organism is prevented by incorporating an antiserum in the medium; this phenomenon has certain similarities to the neutralization of a virus. Growth of bacteria in the L-phase is inhibited by antiserum, but bacteria in the bacillary phase do not appear to be inhibited.

The evidence thus appears to support the opinion of Sabin (1941*a*) that the pleuropneumonia group of organisms belong to a class distinct both from bacteria and viruses, although they have properties in common with both these classes. They appear to be particularly closely related to bacteria through the L-phase variants of the latter. Dienes & Weinberger (1951) suggested that pleuropneumonia-like organisms might have evolved from bacteria by stabilization of L-phase variants, but, since the bacillary to L-phase variation has been shown to be reversible with many bacteria, it could be suggested that bacteria were derived from pleuropneumonia-like organisms which now represent a persistence of a form of micro-organism found early in evolution.

REFERENCES

- ANDREWES, C. H. & WELCH, F. V. (1946). A motile organism of the pleuropneumonia group. *J. Path. Bact.* **58**, 578.
- BEEUWKES, H. & COLLIER, W. A. (1942). Studies on arthrotropic pleuropneumonia-like micro-organisms. *J. infect. Dis.* **70**, 1.
- BEVERIDGE, W. I. B. (1941). Foot-rot in sheep: a transmissible disease due to infection with *Fusiformis nodosus* (n.sp.). *Bull. Coun. sci. industr. Res. Aust.* no. 140.
- BEVERIDGE, W. I. B. (1943). Isolation of pleuropneumonia-like organisms from the male urethra. *Med. J. Aust.* **2**, 479.
- BEVERIDGE, W. I. B., CAMPBELL, A. D. & LIND, P. E. (1946). Pleuropneumonia-like organisms in cases of non-gonococcal urethritis in man and in normal female genitalia. *Med. J. Aust.* **1**, 179.
- BORDET, J. (1910). La morphologie du microbe de la péricapnémie des bovidés. *Ann. Inst. Pasteur*, **24**, 161.
- BORREL, A., DUJARDIN-BEAUMETZ, E., JEANTET & JOUAN (1910). Le microbe de la péricapnémie. *Ann. Inst. Pasteur*, **24**, 168.

- BRIDRÉ, J. & DONATIEN, A. (1923). Le microbe de l'agalaxie contagieuse et sa culture *in vitro*. *C.R. Acad. Sci., Paris*, **177**, 841.
- BRIDRÉ, J., DONATIEN, A. & HILBERT, D. (1928). Le stovarsol, spécifique de l'agalaxie contagieuse du mouton et de la chèvre. *C.R. Acad. Sci., Paris*, **187**, 262.
- BROWN, T. McP., WICHELHAUSEN, R. H., ROBINSON, L. B. & MERCHANT, W. R. (1949). The *in vivo* action of aureomycin on pleuropneumonia-like organisms associated with various rheumatic diseases. *J. Lab. clin. Med.* **34**, 1404.
- CAILLEAU, R. (1937). La nutrition des flagellés tétramitidés. Les sterols, facteurs de croissance pour les trichomonades. *Ann. Inst. Pasteur*, **59**, 293.
- CAMPBELL, A. D. (1938). A preliminary note on the experimental production of bovine pleuropneumonia. *J. Coun. sci. industr. Res. Aust.* **11**, 103.
- CARLSON, H. J., SPECTOR, S. & DOUGLAS, H. G. (1951). Possible role of pleuropneumonia-like organisms in etiology of disease in childhood. *Amer. J. Dis. Child.* **81**, 193.
- CLARKE, P. H. & COWAN, S. T. (1952). Biochemical methods for bacteriology. *J. gen. Microbiol.* **6**, 187.
- DIENES, L. (1939). L organism of Klieneberger and *Streptobacillus moniliformis*. *J. infect. Dis.* **65**, 24.
- DIENES, L. (1940). Cultivation of pleuropneumonia-like organisms from female genital organs. *Proc. Soc. exp. Biol., N.Y.* **44**, 468.
- DIENES, L. (1945). Morphology and nature of the pleuropneumonia group of organisms. *J. Bact.* **50**, 441.
- DIENES, L. (1947a). The morphology of the L1 of Klieneberger and its relationship to *Streptobacillus moniliformis*. *J. Bact.* **54**, 231.
- DIENES, L. (1947b). Isolation of pleuropneumonia-like organisms from pathological specimens with the aid of penicillin. *Proc. Soc. exp. Biol., N.Y.* **64**, 165.
- DIENES, L. (1948). The isolation of L type cultures from *Bacteroides* with the aid of penicillin and their reversion into the usual bacilli. *J. Bact.* **56**, 445.
- DIENES, L. & WEINBERGER, H. J. (1951). The L forms of bacteria. *Bact. Rev.* **15**, 245.
- DIENES, L. & MADOFF, S. (1953). Differences between oral and genital strains of human pleuropneumonia-like organisms. *Proc. Soc. exp. Biol., N.Y.* **82**, 36.
- DIENES, L., ROPES, M. W., SMITH, W. E., MADOFF, S. & BAUER, W. (1948). The role of pleuropneumonia-like organisms in genito-urinary and joint diseases. *New Engl. J. Med.* **238**, 509, 563.
- DUJARDIN-BEAUMETZ, E. (1906). Transmission de la péripneumonie des bovidés aux espèces ovine et caprine. *Ann. Inst. Pasteur*, **20**, 449.
- EDWARD, D. G. FF. (1940). The occurrence in normal mice of pleuropneumonia-like organisms capable of producing pneumonia. *J. Path. Bact.* **50**, 409.
- EDWARD, D. G. FF. (1947a). Catarrh of the upper respiratory tract in mice and its association with pleuropneumonia-like organisms. *J. Path. Bact.* **59**, 209.
- EDWARD, D. G. FF. (1947b). A selective medium for pleuropneumonia-like organisms. *J. gen. Microbiol.* **1**, 238.
- EDWARD, D. G. FF. (1950a). An investigation of pleuropneumonia-like organisms isolated from the bovine genital tract. *J. gen. Microbiol.* **4**, 4.
- EDWARD, D. G. FF. (1950b). An investigation of the biological properties of organisms of the pleuropneumonia group, with suggestions regarding the identification of strains. *J. gen. Microbiol.* **4**, 311.
- EDWARD, D. G. FF. (1952). The pleuropneumonia group of organisms and their significance in genital infection. *Brit. J. vener. Dis.* **28**, 89.
- EDWARD, D. G. FF. (1953a). A difference in growth requirements between bacteria in the L-phase and organisms of the pleuropneumonia group. *J. gen. Microbiol.* **8**, 256.
- EDWARD, D. G. FF. (1953b). Organisms of the pleuropneumonia group causing disease in goats. *Vet. Rec.* **65**, 873.

- EDWARD, D. G. FF. & FITZGERALD, W. A. (1951*a*). The isolation of organisms of the pleuropneumonia group from dogs. *J. gen. Microbiol.* **5**, 566.
- EDWARD, D. G. FF. & FITZGERALD, W. A. (1951*b*). Cholesterol in the growth of organisms of the pleuropneumonia group. *J. gen. Microbiol.* **5**, 576.
- EDWARD, D. G. FF. & FITZGERALD, W. A. (1952). A growth factor needed to isolate organisms of the pleuropneumonia group from the genital tract of cattle. *Vet. Rec.* **64**, 395.
- EDWARD, D. G. FF. & FITZGERALD, W. A. (1954). Inhibition of growth of pleuropneumonia-like organisms by antibody. *J. Path. Bact.* (in the Press).
- EDWARD, D. G. FF., HANCOCK, J. L. & HIGNETT, S. L. (1947). Isolation of pleuropneumonia-like organisms from the bovine genital tract. *Vet. Rec.* **59**, 329.
- ELFORD, W. J. (1929). Ultrafiltration methods and their application in bacteriological and pathological studies. *Brit. J. exp. Path.* **10**, 26.
- ELFORD, W. J. (1938). The size of viruses and bacteriophages, and methods for their determination. In *Handbuch der Virusforschung*, edited by R. Doerr & C. Hallauer, Vienna, **1**, 126.
- FINDLAY, G. M. (1946). Pleuropneumonia-like organisms and arthritis. *Ann. Rheum. Dis.* **5**, 153.
- FINDLAY, G. M., KLIENEBERGER, E., MACCALLUM, F. O. & MACKENZIE, R. D. (1938). Rolling Disease. New syndrome in mice associated with a pleuropneumonia-like organism. *Lancet*, ii, 1511.
- FINDLAY, G. M., KLIENEBERGER, E., MACCALLUM, F. O. & MACKENZIE, R. D. (1939*a*). Eperythrozoon in the blood of mice and its possible relationship to pleuropneumonia-like organisms. *Trans. R. Soc. trop. Med. Hyg.* **33**, 6.
- FINDLAY, G. M., MACKENZIE, R. D., MACCALLUM, F. O. & KLIENEBERGER, E. (1939*b*). The aetiology of polyarthritis in the rat. *Lancet*, ii, 7.
- FINDLAY, G. M., MACKENZIE, R. D. & MACCALLUM, F. O. (1940). Chemotherapeutic experiments in pleuropneumonia-like organisms in rodents. *Brit. J. exp. Path.* **21**, 13.
- FREUNDT, E. A. (1950). Observations on the Dienes L type growth of bacteria. *Acta path. microbiol. scand.* **27**, 159.
- FREUNDT, E. A. (1952*a*). Morphological studies of the peripneumonia organism (*Micromyces peripneumoniae bovis*). *Acta path. microbiol. scand.* **31**, 508.
- FREUNDT, E. A. (1952*b*). The nature of the 'large bodies' in the peripneumonia organism (*Micromyces peripneumoniae bovis*). *Acta path. microbiol. scand.* **31**, 561.
- GILMORE, E. L. & SPRINCE, H. (1949). The growth of human pleuropneumonia-like organisms in a simplified fluid medium. *J. Bact.* **57**, 473.
- GRÜNHOLZ, G. (1950). Pleuropneumonieartige Mikroorganismen bei 'Viruspneumonien'. *Klin. Wschr.* **28**, 480.
- HARKNESS, A. H. (1950). *Non-Gonococcal Urethritis*. Edinburgh: Livingstone.
- HARKNESS, A. H. & HENDERSON-BEGG, A. (1948). The significance of pleuropneumonia-like or 'L' organisms in non-gonococcal urethritis, Reiter's disease, and abacterial pyuria. *Brit. J. vener. Dis.* **24**, 50.
- HATCH, M. H. (1949). *A Symposium on Current Progress in the Study of Venereal Diseases*, p. 185. Washington: U.S. Public Health Service.
- HERICK, W. VAN, & EATON, M. D. (1945). An unidentified pleuropneumonia-like organism isolated during passages in chick embryos. *J. Bact.* **50**, 47.
- HOLMES, B. E. (1937). The metabolism of the filter-passing organism 'A' from sewage. *Brit. J. exp. Path.* **18**, 103.
- HOLMES, B. E. & PIRIE, A. (1932). Growth and metabolism of the bovine pleuropneumonia virus. *Brit. J. exp. Path.* **13**, 364.
- KELLER, R., SMITH, P. F. & MORTON, H. E. (1952). Susceptibility of pleuropneumonia-like organisms from human genital tract to the action of soaps. *J. gen. Microbiol.* **7**, 313.

- KLIENEGER, E. (1934). The colonial development of the organisms of pleuropneumonia and agalactia on serum agar and variations of the morphology under different conditions of growth. *J. Path. Bact.* **39**, 409.
- KLIENEGER, E. (1935). The natural occurrence of pleuropneumonia-like organisms in apparent symbiosis with *Streptobacillus moniliformis* and other bacteria. *J. Path. Bact.* **40**, 93.
- KLIENEGER, E. (1936). Further studies on *Streptobacillus moniliformis* and its symbiont. *J. Path. Bact.* **42**, 487.
- KLIENEGER, E. (1938). Pleuropneumonia-like organisms of diverse provenance: some results of an enquiry into methods of differentiation. *J. Hyg., Camb.* **38**, 458.
- KLIENEGER, E. (1939). Studies on pleuropneumonia-like organisms: the L4 organism as the cause of Woglom's 'pyogenic virus'. *J. Hyg., Camb.* **39**, 260.
- KLIENEGER, E. (1940). The pleuropneumonia-like organisms: further comparative studies and a descriptive account of recently discovered types. *J. Hyg., Camb.* **40**, 204.
- KLIENEGER-NOBEL, E. (1945). Pleuropneumonia-like organisms in the human vagina. *Lancet*, ii, 46.
- KLIENEGER-NOBEL, E. (1947). Isolation and maintenance of an L1-like culture from *Fusiformis necrophorus* (syn. *Bact. funduliforme*, *Bacteroides funduliformis*). *J. Hyg., Camb.* **45**, 407.
- KLIENEGER-NOBEL, E. (1949). Origin, development and significance of L-forms in bacterial cultures. *J. gen. Microbiol.* **3**, 434.
- KLIENEGER-NOBEL, E. (1954). The organisms of the so-called pleuropneumonia group. *Biol. Rev.* **29** (in the Press).
- KLIENEGER, E. & STEABEN, D. B. (1937). On a pleuropneumonia-like organism in lung lesions of rats, with notes on the clinical and pathological features of the underlying conditions. *J. Hyg., Camb.* **37**, 143.
- KLIENEGER, E. & STEABEN, D. B. (1940). On the association of the pleuropneumonia-like organism L3 with bronchiectatic lesions in rats. *J. Hyg., Camb.* **40**, 223.
- KLIENEGER, E. & SMILES, J. (1942). Some new observations on the developmental cycle of the organism of bovine pleuropneumonia and related microbes. *J. Hyg., Camb.* **42**, 110.
- KUZELL, W. C., GARDNER, G. M. & FAIRLEY, D. M. (1949). Aureomycin in experimental polyarthritis with preliminary trials in clinical arthritis. *Proc. Soc. exp. Biol., N.Y.* **71**, 631.
- KUZELL, W. C. & MANKLE, E. A. (1950). Cortisone acetate and terramycin in polyarthritis of rats. *Proc. Soc. exp. Biol., N.Y.* **74**, 677.
- LAIDLAW, P. P. & ELFORD, W. J. (1936). A new group of filterable organisms. *Proc. Roy. Soc. B*, **120**, 292.
- LEBERMAN, P. R., SMITH, P. F. & MORTON, H. E. (1950). The susceptibility of pleuropneumonia-like organisms to the *in vitro* action of antibiotics: aureomycin, chloramphenicol, dihydrostreptomycin, streptomycin and sodium penicillin G. *J. Urol.* **64**, 167.
- LEBERMAN, P. R., SMITH, P. F. & MORTON, H. E. (1952). Susceptibility of pleuropneumonia-like organisms to action of antibiotics. II. Terramycin and neomycin. *J. Urol.* **68**, 399.
- LEDINGHAM, J. C. G. (1933). The growth phases of pleuropneumonia and agalactia on liquid and solid media. *J. Path. Bact.* **37**, 393.
- LONGLEY, E. O. (1951). *Contagious Caprine Pleuropneumonia. A Study of the Disease in Nigeria*. Colonial Research Publications, no. 7. London: H.M. Stationery Office.
- LOPEZ & LOPEZ (1952). Agalaxie contagieuse. Culture du virus et vaccination préventive et curative. *Bull. Acad. vét. Fr.* **25**, 23.

- MARKHAM, F. S. & WONG, S. C. (1952). Pleuropneumonia-like organisms in the aetiology of turkey sinusitis and chronic respiratory disease of chickens. *Poult. Sci.* **31**, 902.
- MELANIDI, C. (1951). Agalaxie contagieuse des brébis et des chèvres. La forme oedematogène des caprins de Sparte. *Bull. Off. int. Épizoot.* **36**, 363.
- MELÉN, B. (1951). The susceptibility of pleuropneumonia-like organisms to the *in vitro* action of some antibiotics. *Acta path. microbiol. scand.* **30**, 98.
- MELÉN, B. & ODEBLAD, E. (1951). Pleuropneumonia-like micro-organisms in the female genito-urinary tract. *Scand. J. clin. Lab. Invest.* **3**, 47.
- MELÉN, B. & ODEBLAD, E. (1952). Pleuropneumonia-like organisms in the genito-urinary tract of healthy women. *Acta dermat.-venereol., Stockh.* **32**, 74.
- MELÉN, B. & LINNROSS, B. (1952). Pleuropneumonia-like organisms in cases of non-gonococcal urethritis in man. *Acta dermat.-venereol., Stockh.* **32**, 77.
- MORNET, P., BALIS, J. & BACHIROU, S. M. (1949). Action des quelques antibiotiques sur le virus péripleumonique bovin. *Bull. Acad. vét. Fr.* **22**, 225.
- MORNET, P., ORUE, J. & MARTY, J. -P. (1951). Note sur le traitement de la péripleumonie bovine par la pénicilline, la streptomycine et certains dérivés sulfamidés. Action comparée avec le novarsénobenzol. *Bull. Acad. vet. Fr.* **24**, 213.
- MORTON, H. E., SMITH, P. F. & KELLER, R. (1952). Prevalence of pleuropneumonia-like organisms and the evaluation of media and methods for their isolation from clinical material. *Amer. J. publ. Hlth*, **42**, 913.
- MORTON, H. E., SMITH, P. F. & LEBERMAN, P. R. (1951). Investigation of the cultivation of pleuropneumonia-like organisms from man. *Amer. J. Syph.* **35**, 361.
- MORTON, H. E., SMITH, P. F., WILLIAMS, N. B. & EICKENBERG, C. F. (1951). Isolation of pleuropneumonia-like organisms from human saliva: a newly detected member of the oral flora. *J. dent. Res.* **30**, 415.
- MOUSTARDIER, G., BRISOU, J. & PERREY, M. (1953). Transformation en bactéries normales d'organismes L isolés par culture à partir d'uréthrites amicrobiennes à inclusions. *Ann. Inst. Pasteur*, **85**, 520.
- NELSON, J. B. (1935). Cocco-bacilliform bodies associated with an infectious fowl coryza. *Science*, **82**, 43.
- NELSON, J. B. (1937). Infectious catarrh of mice. I. A natural outbreak of the disease. II. The detection and isolation of cocco-bacilliform bodies. III. The etiological significance of the cocco-bacilliform bodies. *J. exp. Med.* **65**, 833.
- NELSON, J. B. (1940). Infectious catarrh of the albino rat. II. The causal relation of coccobacilliform bodies. *J. exp. Med.* **72**, 655.
- NELSON, J. B. (1948). The nasal transmission of pleuropneumonia-like organisms in mice and rats. *J. infect. Dis.* **82**, 169.
- NELSON, J. B. (1950a). Association of a special strain of pleuropneumonia-like organisms with conjunctivitis in a mouse colony. *J. exp. Med.* **91**, 309.
- NELSON, J. B. (1950b). The relation of pleuropneumonia-like organisms to the conjunctival changes occurring in mice of the Princeton strain. *J. exp. Med.* **92**, 431.
- NICOL, C. S. & EDWARD, D. G. FF. (1953). The role of organisms of the pleuropneumonia group in human genital infections. *Brit. J. vener. Dis.* **29**, 141.
- NOCARD & ROUX (1898). Le microbe de la péripleumonie. *Ann. Inst. Pasteur*, **12**, 240.
- NORMAN, M. C., SASLAW, S. & KUHN, L. R. (1950). Antigenic similarity of five human strains of pleuropneumonia-like organisms. *Proc. Soc. exp. Biol., N.Y.* **75**, 718.
- NOWAK, J. & LOMINSKI, I. (1934). Morphologie et évolution du microbe de l'agalaxie contagieuse des ovins et des caprins dans les milieux nutritifs. *Ann. Inst. Pasteur*, **53**, 438.
- ØRSKOV, J. (1942). *Streptobacillus moniliformis* and the morphology of its variants. *Acta path. microbiol. scand.* **19**, 575.

- PAINE, JUN., T. F., MURRAY, R., PERLMUTTER, I. & FINLAND, M. (1950). Brain abscess and meningitis associated with a pleuropneumonia-like organism: clinical and bacteriological observations in a case with recovery. *Ann. intern. Med.* **32**, 554.
- PARKES, M. W., WRIGLEY, F. & O'BRIEN, B. (1951). Arthritis in rats produced by pleuropneumonia-like organisms. *Ann. rheum. Dis.* **10**, 177.
- PIRIE, A. (1937). The metabolism of the filter-passing organism 'C' from sewage. *Brit. J. exp. Path.* **18**, 96.
- PIRIE, A. (1938). The effect of catalase on the respiration of a filtrable organism from sewage. *Brit. J. exp. Path.* **19**, 9.
- PIRIE, A. & HOLMES, B. E. (1933). A study of the enzymes of the agalactia virus. *Brit. J. exp. Path.* **14**, 290.
- POWELL, H. M. & RICE, R. M. (1944). Ineffective penicillin chemotherapy of arthritic rats infected with pleuropneumonia-like organisms. *J. Lab. clin. Med.* **29**, 372.
- POWELL, H. M., JAMIESON, W. A. & RICE, R. M. (1946). Effectiveness of streptomycin in arthritis of rats. *Proc. Soc. exp. Biol., N.Y.* **62**, 8.
- PRESTON, W. S. (1942). Arthritis in rats caused by pleuropneumonia-like micro-organisms and the relationship of similar organisms to human rheumatism. *J. infect. Dis.* **70**, 180.
- PRIESTLEY, F. W. (1952). Observations on immunity to contagious bovine pleuropneumonia, with special reference to the bactericidal action of blood. *Brit. vet. J.* **108**, 153.
- PRIESTLEY, F. W. & WHITE, R. W. (1952). A note on the isolation, from serum, of a heat stable growth factor for the contagious bovine pleuropneumonia organism. *Vet. Rec.* **64**, 259.
- PULVERTAFT, R. J. V. (1953). The L form of bacteria in relation to antibiotics. *J. Path. Bact.* **65**, 175.
- RANDALL, J. H., STEIN, R. J. & AYRES, J. C. (1950). Pleuropneumonia-like organisms of female genital tract; study of 300 gynecological cases. *Amer. J. Obstet. Gynec.* **59**, 404.
- ROBINSON, L. B., WICHELHAUSEN, R. H. & BROWN, T. MCP. (1952). Sensitivity studies on human pleuropneumonia-like organisms. *J. Lab. clin. Med.* **39**, 290.
- ROEKEL, H. VAN, OLESIUKE, O. M. & PECK, H. A. (1952). Chronic respiratory disease of chickens. *Amer. J. vet. Res.* **13**, 252.
- RUITER, M. & WENTHOLT, H. M. M. (1952). The occurrence of a pleuropneumonia-like organism in fuso-spirillary infections of the human genital mucosa. *J. invest. Dermat.* **18**, 313.
- SABIN, A. B. (1941a). The filtrable micro-organisms of the pleuropneumonia group. *Bact. Rev.* **5**, 1.
- SABIN, A. B. (1941b). The filtrable micro-organisms of the pleuropneumonia group (Appendix on classification and nomenclature). *Bact. Rev.* **5**, 331.
- SABIN, A. B. & JOHNSON, B. (1940a). Search for micro-organisms of the pleuropneumonia group in rheumatic and non-rheumatic children. *Proc. Soc. exp. Biol., N.Y.* **44**, 565.
- SABIN, A. B. & JOHNSON, B. (1940b). Pathogenic pleuropneumonia-like micro-organisms in tissues of normal mice and isolation of new immunological types. *Proc. Soc. exp. Biol., N.Y.* **44**, 569.
- SABIN, A. B. & WARREN, J. (1940a). The curative effect of certain gold compounds on experimental, proliferative, chronic arthritis in mice. *J. Bact.* **40**, 823.
- SABIN, A. B. & WARREN, J. (1940b). The therapeutic effectiveness of a practically non-toxic new compound (calcium aurothiomalate) in experimental, proliferative, chronic arthritis of mice. *Science*, **92**, 535.

- SEIFFERT, G. (1937*a*). Über das Vorkommen filtrabler Mikroorganismen in der Natur und ihre Züchtbarkeit. *Zbl. Bakt.* (1 Abt., Orig.), **139**, 337.
- SEIFFERT, G. (1937*b*). Filtrabler Mikroorganismen in der freien Natur. *Zbl. Bakt.* (1 Abt., Orig.), **140**, 168.
- SHERIFF, D. & PIERCY, S. E. (1952). Experiments with an avianised strain of the organism of contagious bovine pleuropneumonia. *Vet. Rec.* **64**, 615.
- SHOETENSACK, H. M. (1934). Pure cultivation of the filtrable virus isolated from canine distemper. *Kitasato Arch.* **11**, 277.
- SHOETENSACK, H. M. (1936*a*). Pure cultivation of filtrable virus isolated from canine distemper (Part II). Morphological and cultural features of *Asterococcus canis*, Type I, n.sp. and *Asterococcus canis*, Type II, n.sp. *Kitasato Arch.* **13**, 175.
- SHOETENSACK, H. M. (1936*b*). Studies concerning the relation between canine distemper and *Asterococcus canis* Types I and II. *Kitasato Arch.* **13**, 269.
- SMITH, P. F. & MORTON, H. E. (1951*a*). The separation and characterization of the growth factor in serum and ascitic fluid which is required by certain pleuropneumonia-like organisms. *J. Bact.* **61**, 395.
- SMITH, P. F. & MORTON, H. E. (1951*b*). Isolation of pleuropneumonia-like organisms from the throats of humans. *Science*, **113**, 623.
- SMITH, P. F. & MORTON, H. E. (1952). Further characterization of the protein factor required by certain pleuropneumonia-like organisms for growth *in vitro*. *Arch. Biochem.* **38**, 23.
- SMITH, P. F., MORTON, H. E. & LEBERMAN, P. R. (1950). Susceptibilities of pleuropneumonia-like organisms to some selective bacteriostatic agents. *Proc. Soc. exp. Biol.*, N.Y. **74**, 552.
- SMITH, W. E., HILLIER, J. & MUDD, S. (1948). Electron micrograph studies of two strains of pleuropneumonia-like (L) organisms of human derivation. *J. Bact.* **56**, 589.
- SULLIVAN, E. R. & DIENES, L. (1939). Pneumonia in white mice produced by a pleuro-pneumonia-like micro-organism. *Proc. Soc. exp. Biol.*, N.Y. **41**, 620.
- SWIFT, H. F. (1941). Capacity of pleuropneumonia-like micro-organisms to grow on chorioallantoic membranes. *J. exp. Med.* **74**, 557.
- SWIFT, H. F. & BROWN, T. MCP. (1939). Pathogenic pleuropneumonia-like organisms from acute rheumatic exudates and tissues. *Science*, **89**, 271.
- SZABÓ, L. (1951). Improved sampling method for demonstration of local antibodies in the vagina. *Nature, Lond.* **168**, 171.
- TANG, F. F., WEI, H., MCWHIRTER, D. L. & EDGAR, J. (1935). An investigation of the causal agent of bovine pleuropneumonia. *J. Path. Bact.* **40**, 391.
- TANG, F. F., WEI, H. & EDGAR, J. (1936). Further investigations of the causal agent of bovine pleuropneumonia. *J. Path. Bact.* **42**, 45.
- TULASNE, R. (1951). Les formes L des bactéries. *Rev. Immunol. Paris*, **15**, 223.
- TURNER, A. W. (1935*a*). A study on the morphology and life cycles of the organism of *Pleuropneumonia contagiosa boum* (*Borrelomyces peripneumoniae* nov.gen.) by observation in the living state under dark-ground illumination. *J. Path. Bact.* **41**, 1.
- TURNER, A. W. (1935*b*). *Pleuropneumonia contagiosa boum*: the staining of the causal organism in the specific lesions. *Aust. J. exp. Biol. med. Sci.* **13**, 149.
- VISHNIAC, S. & WATSON, S. W. (1953). The steroid requirements of *Labyrinthula vitellina* var. *pacifica*. *J. gen. Microbiol.* **8**, 248.
- WALKER, J. (1930). *Pleuropneumonia contagiosa bovum*. *A System of Bacteriology in Relation to Medicine*, Vol. 7. Medical Research Council. London: H.M. Stationery Office.
- WARREN, J. (1942). Observations on some biological characteristics of organisms of the pleuropneumonia group. *J. Bact.* **43**, 211.
- WEISS, L. J. (1944). Electron micrographs of pleuropneumonia-like organisms. *J. Bact.* **47**, 523.

- WHITE, R. W. (1952). Selective bacteriostatic substances for the isolation and cultivation of *Asterococcus bovis*, the causal organism of contagious bovine pleuropneumonia. *Brit. vet. J.* **108**, 111.
- WROBLEWSKI, W. (1931). Morphologie et cycle évolutif des microbes de la péri-pneumonie des bovidés et de l'agalaxie contagieuse des chèvres et des moutons. *Ann. Inst. Pasteur*, **47**, 94.
- WYCKOFF, R. W. G. (1953). Formation of the particles of influenza virus. *J. Immunol.* **70**, 187.
- ZAVAGLI, V. (1951). L'agalaxie contagieuse des brébis et des chèvres. *Bull. Off. int. Épizoot.* **36**, 336.

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ADDENDUM

Moustardier, Brisov & Perry (1953) have claimed that 4 of 13 strains of pleuropneumonia-like organisms, isolated from the human genital tract, when subcultured on ordinary media, changed into bacteria, two strains into *Streptococcus faecalis*, one into *Proteus mirabilis* and one into *Bacterium faecalis alkaligenes*. These findings were adduced as evidence that the genital pleuropneumonia-like organisms are the stabilized L-phase of various bacteria and it was suggested that the strains which did not revert were possibly the L-phase of the gonococcus. In view of the significance of these claims, which are difficult to reconcile with the findings of Nicol & Edward (1953) that all except one of 91 strains of pleuropneumonia-like organisms, isolated from the genital tract, were serologically similar, it is unfortunate that the authors give few experimental details. The biological and serological properties of the pleuropneumonia-like organisms were apparently not examined for comparison with those of the bacteria into which the organisms appeared to change. Moreover, it is impossible to determine from the report whether the original colonies were typical of organisms of the pleuropneumonia group or had the colonial characteristics shown by L-phase organisms.

Table 1. *Summary of properties of species of the pleuropneumonia group of organisms*

Species or strain	Description	Growth on serum-free media	Production of film and spots	Growth on rabbit serum agar	Growth in semi-solid medium	Granular or smooth	Haemolysis of horse blood agar	Discoloration of horse erythrocytes by supernatant of culture	Fermentation of carbohydrates								Pathogenicity for mice
									Glucose	Fructose	Galactose	Maltose	Mannose	Dextrin	Starch	Glycogen	
<i>Asterococcus mycoides</i>	Causes contagious pleuropneumonia in cattle	—	—	Poor	Better near surface	Smooth	+	+	+	+	—	+	+	+	+	+	None
<i>A. mycoides</i> var. <i>capri</i>	Causes contagious pleuropneumonia in goats	Trace	—	Good	Better near surface	Smooth	+	+	+	+	—	+	+	+	+	+	None
<i>Anulomyces agalaxiae</i>	Causes contagious agalactia in sheep and goats	—	Usually +	Poor	Better near surface	Smooth	+	—	—	—	—	—	—	—	—	—	None
L3	From lungs of rats	—	+	Poor	Better near surface	Granular	+	—	+	—	—	+	+	+	+	+	Abscesses after s/c inoc.
L4	Causes polyarthritis in rats	—	—	Good	Throughout	Smooth	+	—	—	—	—	—	⊙	—	⊙	⊙	Diffuse abscesses after s/c inoc.
L5	From mice	—	—	Poor	Better near surface	Granular	+	—	+	—	—	+	+	+	+	+	Originally produced 'rolling disease' after intracerebral inoc.
Strain M50*	From mice with infectious catarrh	—	+	Good	Better near surface	Smooth becoming granular with subcultivation	+	⊙	+	±	? ±	+	+	+	+	+	Early subcultures produced catarrh and pneumonia by nasal instillation; later abscesses after s/c inoc.
P*	From genital tract of cattle	—	+	Poor	Throughout	Smooth	+	—	—	—	—	—	⊙	—	⊙	⊙	None
α	From genital tract of dogs	—	—	Poor	Throughout	Indefinite	Trace	⊙	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙	Not tested
β	From throat and genital tract of dogs	—	—	Good	Throughout	Indefinite	+	⊙	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙	Not tested
γ	From throat and genital tract of dogs	—	+	Poor	Throughout	Indefinite	±	⊙	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙	Not tested
Fowl coryza	From upper respiratory tract of fowls	—	+	Good	Throughout	Smooth	+	⊙	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙	Not tested
Human type 1	From genital tract and anal canal	—	—	Good	Throughout	Usually granular	— or trace	—	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙	None
Human type 2	From genital tract	—	—	Good	Throughout	Usually granular	—	⊙	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙	Abscesses after s/c inoc.
Human type 3*†	From ulcerative lesions of glans penis	—	+	Good	At bottom only	Smooth	—	⊙	+	+	—	+	—	⊙	+	+	Early subcultures produced abscesses after s/c inoc.
Strain H106†	From human urethra	—	+	Good	At bottom only	Indefinite	—	⊙	+	+	—	+	—	+	+	+	Not tested
Human type 4*†	From saliva	—	+ or —	Very good	At bottom only	Smooth	—	⊙	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙	Not tested
Saprophytic strains	From sewage and from samples taken from genital tract of cattle	+	—	⊙	Better near surface	Smooth	+	—	+	+	+ or —	+	—	+	+	+	None

* Growth improved by thymus nucleic acid at first isolation.

† Strains grown anaerobically.

⊙ = not tested.