7. Summary.

1. The virus of contagious bovine pleuro-pneumonia can be cultivated in serum peptone broth, and in this medium gives a richer culture than in "Martin's broth." It also grows very well on solid media made by adding agar to the above. It is recommended that 2 per cent. of peptone be used instead of the 1 per cent. employed in the commoner bacteriological media.

2. In the above liquid medium the virus becomes harmless after about six weekly subcultures. At this stage no visible reaction of any kind is noticed in test cattle.

3. Cultures of few generations in subculture produce immunity more rapidly than those of many generations.

4. No "reaction" is necessary in a vaccinated beast in order to ensure immunization. A single injection of a small volume of culture of the correct degree of attenuation is adequate.

5. Few observations have been made on the duration of immunity, but two cattle were found to be immune after one year and seven months.

6. In the field, vaccinations carried out on a basis of the foregoing principles have been very satisfactory. The only apparent objection is the occasional development of large swellings, and these seem not to be referable to the nature of the vaccine, but to the difficulty of skin sterilization under field conditions.

Paper No. 14.

PLEURO-PNEUMONIA CONTAGIOSA BOVUM.

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(Bovine Pleuro-pneumonia: Lung-sickness of cattle, British; Lungenseuce der Rinder, German; Peripneumonie contagieuse, French; Polmonera-polmonite essudativa, Italian.)

Bovine pleuro-pneumonia is a contagious disease naturally confined to bovines; it is caused by a filterable virus which produces an exudative fibrinous pneumonia and pleuritis, and specific histological lesions.

GEOGRAPHICAL DISTRIBUTION.

It is said that bovine pleuro-pneumonia occurred from time immemorial in Central Europe. It remained localized in a portion of Germany, Switzerland, France, and Italy, up till the end of the 17th century, and subsequently extended in various directions. About the middle of the 19th century it had invaded the countries of Western Europe, and as the result of the exportation of infected cattle was introduced into other parts of the world.

From the literature at disposal, bovine pleuro-pneumonia still occurs in parts of Germany, Poland, the Balkans (Roumania), Spain, Russia-in-Asia, India, South America, Australia, Japan, China, and Korea. In Africa, it is known to occur in the Sudan; Senegal; Nigeria; the Central-East African territories, viz., Kenya, Uganda, and Tanganyika; Northern Rhodesia; Bechuanaland; Portuguese East Africa; Barotseland, and probably exists in other remote parts of the African continent.

HISTORICAL.

The more important steps by which our knowledge of the disease has been advanced have been briefly referred to.

Bourgelat (1765) was the first to give a good description of bovine pleuro-pneumonia; many of the older authors were of opinion that it was of spontaneous origin, others considered it to be a typhoid or gangrenous affection, while others held the view that it was a simple paralysis of the lung.

Chabert (1794) insisted on its contagiousness, but up till 1846, opinions differed thereon. Delafond (1840) established the different methods of transmission and dissemination of infection and investigations of commissions appointed in the middle of the nineteenth century substantiated their views. Willems (1852) recorded results of observations which confirmed its contagiousness, and established that the pulmonary or pleural serosity when inoculated subcutaneously to susceptible cattle produced a specific local reaction and conferred an immunity against natural infection, and thus originated a method of immunization which, with some modifications, is employed up to the present day.

Nocard and Roux (1898) with the collaboration of Borrel, Salimbeni and Dujardin-Beaumetz were the first to demonstrate causal agent of bovine pleuro-pneumonia.

During the following years, Nocard, Roux and Dujardin-Beaumetz (1899) (1901) completed the study of the microbe.

Dujardin-Beaumetz (1900) described its filterability through the Berkefeld and Chamberland F filters; its cultivation *in vitro* in liquid and on solid media, and recorded his success in experimentally producing by subcutaneous inoculation of pure culture a specific local reaction.

Dujardin-Beaumetz (1906) obtained in other species (sheep and goats) by subcutaneous inoculation of large doses of pure culture of the organism in Martin's peptone, sheep or beef bouillon additioned with sheep or horse serum, similar lesions at the seat of inoculation to those occuring in cattle inoculated with pure culture in Martin's peptone beef bouillon additioned with ox serum.

The morphology of the organism was investigated and described by Bordet (1910); Borrel, Dujardin-Beaumetz, Jeantet and Jouan (1910); Martzinovski (1911); Frosch (1922); Frosch (1923); Orskov (1927).

The following are some of the many workers who have contributed to our knowledge of the pathological-anatomical appearances and histological lesions which occur in bovine pleuro-pneumonia: Furstenburg (1867); Sussdorf (1879); Pourcelot (1881); and MacFadyean (1892); Csokor (1898); Meyer (1909); Ziegler (1921); Seifried (1926).

Researches into the serum diagnosis of bovine pleuro-pneumonia were carried out by different workers, e.g., Dujardin-Beaumetz (1900) (1906); Schowkosky (1912); Poppe (1913); Meyer (1914); Titze and Giese (1919); Titze, Giese and Wedemann (1923); Heslop (1920) (1921); Dahmen (1922); Seeleman (1923); Walker (1923); Dahmen (1923); Nakamura, Futamara and Watimiki (1926).

The hyper-sensitiveness or otherwise of infected animals to the injection of the organism or its products (allergic reaction) was investigated by Siedamgrotsky and Noack (1892); Walter (1892): Beitzen (1919); Titze, Giese and Wedemann (1923).

MORPHOLOGY AND STAINING.

(1) Morphology. Although the morphological appearance described by various workers corresponds in some respects, and opinions differ as to the group in which the organism should be classed, it is generally accepted that the causal agent of bovine pleuro-pneumonia presents variations in size and shape giving rise to polymorphism. The rapidity with which the various forms develop in liquid culture, the medium in which it is grown, the age of the culture, and the methods employed for examination would appear to explain the dissimilarity.

The morphological appearances described by Borrel, Dujardin-Beaumetz, Jeantet and Jouan (1910), are generally accepted and the scientific name *Asterococcus mycoides* proposed by these investigators for the organism of bovine pleuro-pneumonia is generally looked upon as representing its principle characters. They found the morphology was influenced by the age of the culture and that the various forms succeeded each other more or less rapidly in chronological order, that the spirillum or vibrio forms described by Bordet (1910) are only one of the aspects which the organism takes; that the organism is ensheathed within a mucin matrix, visible by subcolouration, and occurs in diplococci, chain and mass formation in which the individuals are of different size; in the two latter, each unit can divide originating bifurcations and asteroid forms; tripolar forms are frequent and more so the bipolar or pseudo-vibrio forms. The arrangement of individual organisms in ring formation is frequent, the ring can lengthen in the form of a filament or chain. In some 3-4 days old preparations the pseudo-vibrio form is dominant. There also occur masses of granulations in tetrads and mulberry shape which can bud and give rise to filaments, this stage being the commencement of the asteriod forms. The filamentous forms are constant in 5-6 days' old cultures. In very old cultures the more extraordinary forms of involution and coalescence of mucin substance secreted by the older individuals are to be seen.

Bordet (1910) observed in giemsa stained smears of pure culture in a medium consisting of rabbit blood agar containing a little of extract of potato and glycerine, filaments of different shapes, viz., curved flexuous undulations, S or spiral, resembling somewhat a vibrio or spirochaete, and roundish granulations.

In a 24-28 hours' old culture in liquid media consisting of bouillon peptone and fresh serum or blood of the rabbit, filaments, a little shorter, and rare forms in Yoccu, globular forms and granulations with a clear centre were not frequent but they become more numerous as the culture ages; the author concluded that the

globular forms originate from the filaments and transformation takes place in from two to ten days recalling that which occurs in old cultures of the cholera vibrio, but does not consider they are "involution" or "attenuated" forms and questions whether they are "resistant" forms.

Martzinovski (1911) microscopically examined Giemsa stained smears of the organism in pure culture collected after centrifuging, and unfixed Giemsa stained smears of hepatized lung.

He considers that the organism of bovine pleuro-pneumonia should be included in the cocco-bacillary group.

Titze and Seeleman (1921) examined cultures in the dark field and found the organism presented the following aspect, viz., numerous roundish refractile bodies of different size showing molecular movement sometimes two or three lying together as if bound together by an invisible bridge, with the movement in the same direction; ring forms with clear periphery and dark centre being often visible, filamentous or branching forms were not seen; small bodies were also observed in the culture and in sterile Martin's broth and serum which they conclude are non-specific. They demonstrated in support of their views that the refractile bodies were not according to Freiberger (1912) reaction products, their agglutinability by the serum of a hyper-vaccinated beast and the specific local reaction produced when inoculated to susceptible cattle.

Frosch (1922) working with ultra-violet light found that the organism, viz., oval or polygonal in shape, measures from 0.2 to 0.8 microns in diameter, but left its nature undetermined and in 1923 described the appearances presented by the organism in a number of different culture media.

Orskov (1927) describes the primary form of the virus as a small oval or short rod-shaped element which gives rise to a ramified mycelium. The virus had a tendency to autolyse and form elements which vary in shape and which have lost their power of germination.

(2) STAINING. Asterococcus mycoides is gram negative. In bouillon cultures it stains with an aqueous or, preferably, carbolized solution of the aniline dyes.

Giemsa stain is preferred by some.

Bordet (1910) uses Giemsa stain in the proportion of 5 drops to 2 ccs. of distilled water, heated on the slide for 1-2 minutes, Borrel, Dujardin-Beaumetz, Jeantet and Jouan (1910) found with Giemsa many of the forms escape detection and are indefinable. They prefer Leoffler's Mordant method for studying the morphology and make smear preparations of the organisms which accumulate at the bottom of the tube on centrifuging a liquid culture.

They found smear preparations of cultures on solid media unsuitable owing to the difficulty of removing the organism free of serosity in a recent culture, and of eliminating the substance which ensheathes the organism in older cultures.

Bordet (1910) found gentian violet leaves too much deposit and that with blue de toluidine the filaments are not stained but appear as bluish points.

CULTIVATION *IN VIVO*.

Nocard and Roux (1898) with the collaboration of Borrel, Salimbeni, and Dujardin-Beaumetz successfully cultivated the organism by inserting in the peritoneal cavity of rabbits, collodion sacs containing bouillon inoculated with a few drops of sero-fibrinous exudate collected from the pleural cavity of a naturally infected beast. Although with a high magnification (1,500-2,000 diameters), and high illumination a number of small bodies were observed in the contents of the sacs their structure could not be accurately determined, but their specificity was demonstrated by inoculation of susceptible cattle.

CULTIVATION *IN VITRO*.

(1) In Liquid and (2) on Solid media.

Development takes place between 30° and 42° Cent.; the optimum temperature lies between 36° and 38° Cent.; below 30° Cent. no growth takes place.

(1) In liquid media.—The medium commonly employed consists of Martin's peptone beef bouillon additioned with bovine serum brought to Ph. 7.5.

Growth occurs in beef bouillon, or Martin's peptone beef bouillon, additional with either of the following sera, viz., human, horse, swine, sheep, goat, rabbit, and fowl as well as with escitic fluid. Nakamura, Futamara and Watimiki (1926) prepared broth from beef, pork, horse, and rabbit flesh, but cultural experiments failed to reveal any marked superiority of these.

The addition of 0.5 per cent. glucose increases growth.

Serum requirements.

The addition of serum to the medium is essential. With less than 5 per cent. growth is usually scanty. The addition of large quantities 20 per cent. and over to liquid media produces an opalescence which is likely to overmask the growth.

The writer adds 10 per cent. of fresh bovine serum, or horse serum, previously sterilized by filtration through the Berkefeld.

Titze, Giese and Wedemann (1923) found in a liquid media additioned with 7-10 per cent. horse serum the organism develops quicker and better than in such additioned with bovine, sheep or goat serum. They conclude that in a Martin's peptone bouillon serum with an acid reaction a growth scarcely occurs, in a neutral Martin's peptone bouillon serum only a sparing growth, and in a distinctly alkaline the growths is better than in a weak alkaline medium.

For antigen they prefer beef bouillon additioned with horse serum brought to Ph. = 7.8-8.0.

Oxygen requirements.

In cultures, Asterococcus mycoides develop best under aerobic conditions.

The writer finds that a free access of air is not indispensable, that no appreciable influence on growth occurs when bottles containing Martin's peptone beef bouillon bovine serum are corked and sealed with paraffin wax immediately after inoculation of the medium with pure culture and then incubated.

Titze, Giese and Wedemann (1923) record that sealing of the tubes by heat, or liquid paraffin, or vaseline prior to incubation was without essential influence on development and keeping the tubes in an exsiccator and removing the air by suction until the manometer registers 60 cm. did not affect growth. They found that whereas a pressure of 2.6-3.3 atmospheres did not affect development, no growth had occurred at seven days in tubes of culture under oxygen at a pressure of 2.6-3.3 atmospheres, but when the latter were brought under normal atmospheric pressure, a characteristic growth occurred in two days. They consider the above results show that a surplus of oxygen under pressure of 2.6-3.3 atmospheres hinders growth, and that the virus requires also a certain proportion of oxygen, an excess or lack of which prejudices or hinders growth. No development occurred when the inoculated tubes were placed under strong anaerobic conditions (Buchner's method) and the air removed by suction; the cultures were found avirulent after the 6th day.

In a nitrogen, hydrogen or carbonic acid saturated atmosphere, no development had occurred at the 5th day, but when brought under normal atmospheric conditions a characteristic growth occurred within 2-3 days.

Nakamura, Futamura and Watamiki (1926) found that upon solid media the virus grows more readily under aerobic than anaerobic conditions. They further found that it was distinctly advantageous to close their culture tubes with rubber stoppers instead of with cotton wool plugs.

Character of the culture in Martin's peptone beef bouillon additioned with bovine or horse serum.

The Martin's peptone beef bouillon is brought to Ph. = 7.5, distributed in flasks or tubes, autoclaved at a temperature of 108° Cent. for 20 minutes at a steam pressure of 5 lbs. to the square inch and then filtered hot through filter paper (Chardin) and distributed in flasks, tubes, or bottles, leaving space for the addition, after further sterilization, of 10 per cent. serum. The flasks and their contents are now autoclaved for one hour on three consecutive days in flowing steam, and when cold the necessary quantity of serum added by means of a graduated cylinder connected to the flask containing the filtered serum.

Sterility is determined by incubating the flasks, tubes or bottles containing the above medium, at 37° Cent. for a week and if found sterile they are inoculated with a drop or two of pure culture and incubated at 37° Cent., any which show a growth within 36 hours are discarded.

Sterilization of the medium is affected in some laboratories by filtration through the Berkefeld.

After two or three days incubation at 37° Cent.: a slight opalescence appears in the inoculated culture medium; during the course of the next day or two, the opacity becomes more marked and on shaking the tube of culture a characteristic phenomenon is visible, namely, the opalescent fluid takes the form of silkylike moving waves.

Marino's method (1905) is of assistance for determining whether the tubes contain a culture of the organism.

Walker (1921) prepares pure culture vaccine in bulk by the above described method.

(2) On Solid Medium.—The medium commonly employed consists of Martin's peptone beef bouillon serum agar, or beef bouillon serum agar. The medium is first added to the tubes, the tubes then laid in a horizontal position until the contents solidify, and the water of condenzation removed with a pipette. The serum is then added to the surface of the slope, and the tubes incubated for two days in such a position that the serum spreads over the surface and dries.

Character of the culture on solid medium.

After four to five days' incubation at 37° Cent. small single colonies visible with the hand-lens are to be seen. With a low power lens, fine transparent separate colonies which individually have been described as resembling a drop of dew appear on the surface of the slope, they measure approximately 2/10th m.m. in diameter, and have a granular and brownish appearance and a dark central position. The growth extends outwards from the periphery of the colonies which reach a diameter of 1 m.m. The colonies have an *umbilicate* appearance, the central portions grow down into the substance of the medium. With the naked eye the colonies appear whitish. Difficulty is experienced in removing the colonies from the medium owing to their being held together by a viscid material, and to growth extending into the medium.

Dahmen (1922) in earlier experiments found that growth only occurred on the upper third of the surface of the medium where the serum had dried on the surface, but later obtained cultures by mixing the serum with the agar, while melted, and allowing the tubes to set.

BIOCHEMICAL REACTIONS.

1. Action on the Sugars.

In cultures additioned with one per cent. of the sugars, glucose, maltose, dextrin and levulose are attacked, but not lactose, saccharose, mannite, dulcite, arabinose, raffinose, rhaminose, salician, xylose, adonite, galactose, insulin, mannose or sorbite.

2. Acid and Gas Formation.

An acid reaction using Andrade's indicator was apparent with dextrin on the 7th day, with glucose and maltose on the 8th day and with levulose on the 10th day of incubation, but no gas production occurred with any of the sugars.

Titze, Giese and Wedemann (1923) recorded observations on the hydrogen-ion concentration in culture in Martin's peptone bouillon additioned with grape or cane sugar; they found that in 27 days the Ph. of the culture had decreased from 7.8 to 5.8; in Martin's peptone beef bouillon additioned with bovine or horse sera little acidity was produced in an eight days' old culture. They remark that in the medium with a high Ph. at the commencement of growth there is sufficient alkalinity to neutralize the acidity formed by growth.

In bouillon additioned with 28 days' old horse serum and 24 hours' old horse serum respectively and brought to Ph. = 7.5 the Ph. after 30 days' growth which was 7.3 in the first two to three days, had only decreased to 7.2 and then remained constant for 41 days. Cultures were still active on the 30th day.

They found the acid formation is not greater in the higher sugar-containing horse serum than in the sugar-poor bovine serum.

(3) Indol Production.

No formation of Indol occurs in culture.

BIOLOGY.

Vitality—Virulence and Resistance.

On referring to the literature it is observed that usually no distinction is clearly made between the vitality and virulence of the virus.

Asterococcus mycoides does not retain its vitality in liquid culture in tubes plugged with cotton wool and kept at room temperature excluded from strong light, for more than a few weeks. In liquid media containing levulose or maltose, and particularly in glucose, more marked acidity occurs and the cultures become inactive sooner than in liquid media not additioned with sugar.

Dujardin-Beaumetz (1913) records that it is difficult to obtain a growth from a culture in liquid medium after four weeks and when the medium is additioned with glucose, after three weeks. Titze and Giese (1923) found that a 27 days' old culture in Martin's peptone bouillon additioned with grape sugar with a Ph. – 5.8 produced a characteristic growth whereas a 41 day old culture Ph – 5.8 had lost its virulency.

Dahmen (1922) found that cultures on solid medium were visible up to two months.

Titze, Giese and Wedemann (1923) obtained a growth from cultures, in Martin's peptone beef bouillon additioned with bovine serum, kept at room temperature in the dark in tubes plugged with cotton wool, for 59 days but not in cultures kept for 80 days; in tubes plugged with cotton wool sealed with paraffin wax and in tubes hermetically sealed in the flame cultures were still active at six weeks.

A 62 days' old culture, in Martin's peptone bouillon serum on which liquid paraffin was placed on the surface of the culture, produced a characteristic growth in 48 hours.

Nakamura, Futamura and Watamiki (1926) record that cultures kept at 37-38° Cent. had lost their virulence in from 1-2 months; at 5-32° Cent. the virulence was maintained for 2-4 months, and at 3-15° Cent. for a little longer; variations occurred in different cultures and it was necessary to transplant cultures once a month. They found that pleural exudate kept in the ice-chest at 3-15° Cent. retained its virulence for six months.

The writer found subcultures in Martin's peptone beef buillon bovine serum, which had been incubated for nine days and then stored for 21 days at room temperature in the dark, still virulent.

The determination of the period during which a culture retains its virulency is of practical importance when pure cultures are used for immunization purposes.

Nocard and Leclainche (1903) state that in sealed ampoules at temperature below 12° Cent. the microbe in culture retains its virulency for more than six months.

Walker (1922) found that a 41 days' old culture in Martin's peptone beef bouillon bovine serum was still virulent, and observation by the writer showed that a five days' old 3rd generation sub-culture transported by sea and train produced reactions and some mortality in susceptible cattle 21 days after the date of despatch from the laboratory.

LYMPH VIRUS.

Laquerriere (1890) found that the virulency was maintained for, at least, a year in hepatized lung kept in a frozen condition.

Nocard (1892) conserves lymph virus in bottles corked and kept in the dark, by adding 1 volume of virus, half a volume of 5 in 1,000 carbolic acid and half a volume of glycerine, and when required for use dissolves the powder in glycerine.

Walker (1922) found lymph virus to which glycerine had been added in the proportion of 1 of glycerine to 4 of virus was effective up till, at least, 21 days when stored in the dark at ordinary room temperature.

Titze, Giese and Wedemann (1923) recovered the virus by filtration from material which was in a putrid condition for nine days.

ATTENUATION.

Nocard and Leclainche (1903) state that attempts to attenuate lymph virus had not given any result. They found that the serosity when collected purely and sealed in glass tubes conserves its virulency for about one month and the virulence then decreases; in contact with the air and light the virus was already very attenuated after 20-25 days.

Mollereau and Nocard (1903) diluted lymph virus in distilled water (1-50) which did not affect its activity.

Arloing and Rossignol (1903) state the majority of cattle inoculated with serosity heated at 40° Cent. received a certain immunity and that lymph virus when heated at 65° Cent. and 55° Cent. provokes only some very attenuated reactions.

Walker (1922) found that the original culture and 1st and 2nd generations of sub-culture are as virulent as the virus from which they originated; at about the 25th generation of sub-culture the virus is attenuated and produces less marked reactions; from about the 25th to 42nd generation of sub-cultures, the virulency remains constant. The virus though retaining its vitality eventually becomes so attenuated in virulency that it produces few, if any, reactions and it is necessary to discontinue the use of the too attenuated pure culture vaccine and substitute a more virulent sub-culture of another strain, or increase the virulency of the same strain by passage through susceptible cattle before it becomes too attenuated.

DESICCATION.

Titze, Giese and Wedemann (1923) soaked silk threads and glass beads, respectively, in a 48 hours' old culture and dried them for 60 hours over calcium chloride at room temperature in the dark; the former produced a visible growth in 48 hours, the latter in 96 hours in Martin's peptone beef bouillon serum. After being kept in a dried state in sealed tubes for five months the virus was found still active in the silk threads, but only in some of the glass beads. Pieces of cambric cloth soaked in culture and then dried at 37° Cent. for

12 hours as well as pieces dried at 37° Cent. for 15 days, produced a growth in Martin's bouillon serum, whilst after 36 days the virus was inactive.

In a bouillon culture dried in vacuum, over calcium chloride, the virus was still active on the 105th day.

Influence of Dry Heat.

Asterococcus mycoides produces no spores, it is little resistant to heat in tissue as well as in culture. The cultures are readily destroyed in less than an hour by heating at a temperature of 58° Cent.

Nakamura, Futamura and Watamiki (1926) soaked pieces of porous earthenware in culture then dried and exposed them for different periods to varying temperatures (dry heat). 70° Cent. proved fatal in five minutes; 65° Cent. in half an hour; 55° Cent. in one hour; 50° Cent. in two hours, and 45° Cent. in three hours.

Influence of Moist Heat.

They found that cultures when heated in the water-bath were killed in two minutes at 60° Cent.; in five minutes at 55° Cent.; in one hour at 50° Cent.; and four hours at 45° Cent.

(*a*) Natural and (*b*) Experimental Infection.

(*a*) Natural Infection in Cattle.

The history of the spread of bovine pleuro-pneumonia supports the generally accepted view that it is contracted by immediate contact with infected animals; the virus is present, at least in some cases, in the bronchial secretions and nasal discharge, and in the moist exhaled air, and in as much as most filterable viruses on account of their minuteness obey the physical chemical laws which govern the diffusion of gases and of substances in solution they are extremely contagious and possibly air-borne; the virus of bovine pleuro-pneumonia in all probability gains entrance with the inhaled air.

Titze, Giese and Wedemann (1923) conclude that the virus being organotrope, the oxygen in the lung plays a special role in natural infection.

Transmission by an intermediate host has not been established. Outbreaks can usually be traced to the introduction of an infected animal, which presented no clinical symptoms, into the herd.

Walley (1913) found that the virus exists in the encapsulated cavities for 15 months. Minette (1913) traced outbreaks in herds to cattle introduced therein two and three years previously.

Walker (1923) found the virus active, in some instances,, 12 months after the animals were isolated as infected.

Observations in the practice shew that a variable number of susceptible cattle exposed in immediate contact with naturally infected beasts escape infection; the causes of these variations have not so far been explained. Nocard and Leclainche (1903) suggested that they might be due to differences in the activity of the virus and to the resistance of the animal. Walker (1922) found approximately 59 per cent. of non-inoculated susceptible cattle exposed to natural infection in a highly infected herd, for periods varying from approximately three to seven months, did not contract infection. Walker (1922) found that a percentage of cattle subcutaneously

inoculated with the same virus fail to react to an original, but some, at least, react to a subsequent inoculation which would seem to exclude differences in the activity of the virus and support the view that the animal itself is a factor. The difficulty of obtaining a growth in medium with other than an alkaline reaction points to the possibility of the resistance of some animals to natural infection and to inoculation as being due to the reaction of the tissues at time of inoculation.

Further research is considered necessary hereon.

(*b*) Experimental Infection.

(1) *In Cattle.*
(2) *In species naturally refractory.*

(1) Cattle.

Infection by the respiratory tract:

Although infection by inhalation is supported to some extent by the ingenious experiments of Nocard and Roux (1901) and Chauveau (1903), it has not been possible to produce artificially, by inhalation or injection of virulent material, the disease as it occurs under natural conditions. Nocard and Mollereau (1901) did not succeed in transmitting by inoculation of fresh virus into the trachea, and Dujardin-Beaumetz (1913) records non-transmission (one in experiments) by direct inoculation of a virulent culture into the lung itself.

Subcutaneous Inoculation.

In about 6-14 days after the subcutaneous inoculation behind the shoulder of a virulent culture of lymph virus, there occurs in a varying percentage of susceptible cattle a rise of temperature and an oedematous swelling which, in some cases, extends considerably, resulting in the death of the animal in from about 10 to 15 days' time. In other cases, the swelling remains localized and gradually disappears.

In sucking calves subcutaneous inoculation often produces specific lesions in the joints and tendons, viz., synovitis and tendo-vaginitis.

Infection by the Alimentary Canal.

Nocard and Roux (1903) showed that ingestion of large quantities of serosity gave negative results provided the virus did not enter a defect in the tissues. At one time in South Africa drenching with lung serosity was employed for immunizing purposes but was discontinued owing to the accidents which followed. Meyer (1909) described the pathological-anatomy and histological lesions produced in a calf by drenching.

Intravenous Inoculation.

Nocard and Roux (1903) inoculated into the ear vein and immediately after removed a portion of the ear around the site of inoculation so as to exclude the virus developing in the subcutaneous tissue and obtained no reaction and no immunity.

Peritoneum.—Inoculation into the peritoneal cavity produces a marked reaction and a sero-fibrinous exudate usually ending fatally.

Brain.—The inoculation intracerebral of culture, after an incubative period of 6-14 days, produces apathy and drowsiness interrupted by symptoms of cerebral irritation.

Animals refuse food and there is a rapid loss of condition; death occurs in a variable number of days. In young sucking calves there may be no nervous symptoms, but synovitis and tendo-vaginitis may occur as a sequel of inoculation.

Eye.—Inoculation into the anterior chamber gave negative results; inoculated animals are said to have acquired an immunity. Nocard and Leclainche (1903).

Udder.—Inoculation into the udder produces a marked mammitis; within a few days the milk becomes yellowish, caseous and purulent. The oedema extends to the abdomen and may produce death; the pus-like milk contains the virus even after two months and the virus is said to be increased in virulency.

Pleura.—The intrapleural inoculation is followed by an intense inflammation; the lung itself may show hepatization; the peritoneum may become involved by way of the lymph vessels.

(2) Species Naturally Refractory.

Dujardin-Beaumetz (1906) conceived the possibility of experimentally infecting sheep and goats with a virus of bovine origin cultivated in Martin's peptone sheep bouillon additioned with sheep serum, and succeeded in obtaining in sheep and goats by inoculation of from 50-100 c.cs. of the culture a local and temperature reaction analagous to that which occurs in bovines inoculated with lymph virus, or cultures of virus of bovine origin in Martin's peptone beef bouillon ox serum but with this difference, there was an absence of an incubation period in the sheep and goats. He found that cultures in Martin's peptone bouillon sheep serum, possessed a high pathogenicity for the bovine, but virus collected from a bovine reacting to inoculation of a culture in bouillon sheep serum, when cultivated in bouillon serum bovine is non-pathogenic for the sheep and goat; it thus sufficed to substitute the serum of one species for another to overcome the resistance of the naturally refractory species. This investigator then found that the culture in media additioned with the serum of other naturally refractory animals, e.g. the horse, produced a reaction in naturally refractory species, viz., sheep and goats but not in highly susceptible species (bovine), and suggested the utilization of a vaccine for the preventive inoculation of cattle, prepared from cultures of virus of bovine origin in Martin's peptone beef bouillon additional with horse serum with a view to conferring immunity without the risk of losses from inoculation.

In recent experiments unrecorded, the writer found that cultures in Martin's peptone beef bouillon additioned with horse serum produced marked local reactions and some mortality in susceptible cattle.

Beller and Tahssin-Bey (1926) thought it desirable to test the possibility of the transmission of bovine pleuro-pneumonia to small ruminants, because of the possibility of some parallel existing between the spread of pleuro-pneumonia and rinderpest. Outbreaks of the latter occur particularly in the Caucasus, which cannot be explained on any other ground than by means of sheep and goats. Culture media were prepared from the flesh of foetuses additioned with amniotic fluid. Inoculation showed that it was possible to produce infection in both sheep and goats. In pregnant animals the

virus appears to find a suitable medium for the growth of the organism in the foetus, and the disease was limited to the uterus and its contents.

These investigators conclude that, in controlling the disease, the sheep and goat must be kept in mind as possible carriers of the virus.

Camel.—The susceptibility of the camel which Vedernikoff (1893) recorded is very doubtful.

Reindeer.—According to Dschunkowsky (1901) the reindeer can be experimentally infected by subcutaneous inoculation of bovine virus. He obtained, after inoculation, a sero-fibrinous synovitis. Willems (1852) found that rabbits, dogs, fowls, sheep, goats, and swine, do not react to inoculation of bovine lymph virus.

Nocard and Roux (1898) did not succeed in infecting sheep, goats, swine, dogs, rabbits, guinea-pigs and fowls with bovine lymph virus.

Ono (1925) recorded that intraocular inoculation of rabbits produces an iritis and intratesticular inoculation a swelling lasting for 2-3 weeks; there was no suppuration. Serum of the inoculated rabbits gave a precipitin reaction with serum from infected cattle. Walker (1922) found that subcutaneous and intraperitoneal inoculation of virus to small laboratory animals, mice, guinea-pigs and rabbits, and the subcutaneous inoculation of the sheep, the horse, the camel and swine, as well as the intrajugular inoculation of sheep, produced no reaction.

In the writer's experiments unrecorded, rabbits were inoculated intraocular with a culture, but no effect was observed and the serum of the rabbits did not agglutinate a pure culture, nor produce a precipitin reaction in the presence of serum of affected cattle.

PATHOGENESIS.

Different workers have investigated the pathogenesis of the processes which occur consequent on the entry of the virus into the lungs with the inspired air; the conclusions arrived at as regards the primary seat of infection in the lungs, the spread of the virus therein and the first tissue changes sometimes differ.

Sussdorf (1879) recorded an inflammatory process first occurring in one or more places in the interlobular tissue through which the lymph vessels become involved resulting in a serofibrinous infiltration necrosis and neoplastic tissue formation; simultaneously alveolar tissue of the lobules become affected and a fibrinous pneumonia develops; the interlobular and parenchymatous processes extend, the former on the peribronchial and perivascular tissue, the latter on the alveoli and bronchi, there follows a pleuritis serofibrinosa, a more or less wide-spread thrombosis of the arteries, necrosis and sequester formation.

Pourcelot (1881) concluded there is first a pleuritis and subsequently inflammatory changes of the interlobular tissues and later a pneumonia.

Woodhead (1888) found that the virus enters the lymph vessels through the alveoli or bronchi without producing changes therein, and cultivates in the small lymph nodes; the lymph stream subsequently becomes occluded and there follows stasis and involvement of the alveoli and vessels, necrosis and sequester formation.

MacFadyean (1892) concluded that the first changes occur in the inter-alveolar septa and that the alveolar and bronchial changes are secondary.

Csokor (1898) finds that bovine pleuro-pneumonia is a chronic interstitial sequestration pneumonia; that the interstitial tissue is first affected; there occurs a meso and periarteritis of the vessels as a consequence; later the parenchyma becomes involved.

Meyer (1909) is inclined to believe that most pleuro-pneumonia infections originate in the bronchial and peri-bronchial lymph vessels, and spread " per continuitatem " to the rest of the lung and that in the initial stages an " Interstitial pneumonia " due to sero-fibrinous lymphangitis occurs which later involves the neighbouring alveoli; the inflammatory process extends to the lymph spaces of the walls of the blood vessels, producing thrombosis, it being a peculiarity of the virus and its toxins to facilitate the closing of all nutritive channels; the blocking of the vessels give rise to ischaemic necrosis with sequester formation.

Ziegler (1921) found the virus produces a primary bronchitis and bronchiolitis and then invades the surrounding perivascular and peribronchial tissue; inflammatory changes occur within the area in which the lymph vessels are involved; the changes in the interstitia comprise three different stages, viz., inflammatory oedema, necrosis and organization processes (perivascular and marginal), the parenchyma becomes involved when the bronchitic and interlobular changes simultaneously exist, the inflammatory process extends to the pulmonary artery and vein; if the thrombus persists an aputrid anaemic necrosis occurs in the whole of the area supplied by the arteries resulting in sequester formation, in less virulent cases the thrombus disappears and there is organization of the exudate (chronic indurative-pneumonia).

Seifried (1926) concludes that the endobronchi are the primary seat of infection and that the earliest changes occur in the form of lobular sero-cellular seldom fibrinous bronchi pneumonic foci, such may appear as the only changes, from these foci arise either propagation of the inflammatory process with establishment of typical bovine pleuro-pneumonia or the initial and early stages remain limited to their primary focus; in the former case the spread of the virus occurs through the peribronchial (lymphogene) route and leads to involvement of portion of the pleuro and interstitial tissue with inflammatory oedema, necrosis, and organization; the parenchyma becoming involved by the andobronchial (bronchogene) route; both the lymphogene and bronchogene processes keep pace with one another; in consequence of the changes in the perivascular tissue there occurs as a rule thrombosis of the pulmonary blood vessel and resulting necrosis and sequester formation.

Arloing (1921) found a sequester contained within a connective tissue capsule as early as the 50th day.

Walker (1924) observed sequestration and encapsulation had taken place in from 15 to 22 days.

The sequestrum after becoming encapsuled undergoes mummification and remains unaltered for a long time and in which the virus remains active; abscess formation and liquid action may also occur.

The virus does not propagate in the blood stream but may be transported thereby to different parts of the body, viz., in calves the

joints and tendons; metastasis of the virus from the seat of inoculation to other parts of the body, e.g., site of surgical operations, is sometimes observed.

The virus of bovine pleuro-pneumonia evidently exerts its pathogenic action through a toxin; cultivation of the virus in collodion sacs in the peritoneal cavity of a rabbit causes, through its toxin, cachexia and death of the rabbit.

MORBID ANATOMY AND HISTOLOGY.

It is usual to find only one lung affected. In acute cases the hepatized portion which is as a rule conspicuous on account of its roundness and firm consistence may be localized in a portion of a lobe or invade almost the whole of a lobe. On incision a clear yellowish, or yellowish red, fluid exudes from the cut surface and coagulates into a gelatinous-like mass; the interstitial tissue is infiltrated and thickened and separates the hepatized part of the lung into areas varying in size and which according to the stage of hepatization varies in colour, viz.: Dark red, light red, and grey, giving a marbled appearance to the cut surface; the interstitia in the beginning is filled with small cavities containing a clear amber coloured fluid, or fibrinous coagulum, giving a beaded appearance to the septa; in later stages the septa becomes broader and firmer owing to the formation of neoplastic connective tissue, while the lobules lying between are necrotic and may even be calcified.

The walls of the bronchi show serous infiltration and their lumen contains a fibrinous exudate. The blood vessels are thrombosed. The older the stage the more frequent necrosis and sequestration. A sequester appears as a greyish mass surrounded by a capsule of connective tissue.

The pleura is sometimes covered with a fibrinous membrane and there may be no exudate present (pleuritis sicca), in other instances deposits of 1-2 cms. thick are present on the surface, and the pleural cavity contains a clear yellowish or reddish yellow liquid, varying in quantity from one to two litres up to twenty litres. In older cases the pleura is thickened and the lung firmly adherent to the chest wall.

The bronchial and mediastinal glands are enlarged and oedematous. Accessory lesions are sometimes present and consist of a sero-fibrinous pericarditis, the inter-lobular tissue of the liver may be infiltrated and a fibrinous exudate on its surface. In young sucking calves there is frequently a sero-fibrinous synovitis and tendo-vaginitis.

Meyer (1909) recorded this condition in older cattle (cows and oxen). The spleen mediastinal is frequently infiltrated.

Histology.

Meyer (1909) concludes that the virus of bovine pleuro-pneumonia attacks chiefly the connective tissues, viz., interlobular, interalveolar and peribronchial, it causes an inflammatory process which microscopically presents itself as a lymphangitis, sero-fibrinosa, lymphothrombosis, emigration of leucocytes, etc.; the process extending along the lymph vessels involves also the lymph spaces of the walls of the blood vessels resulting in a peri and meso arteritis and thrombus formation. The blocking of the vessels gives rise to necrosis. The lesions of bovine pleuro-pneumonia having originated

by metastasis present microscopically the image of a corpuscular infiltration with pronounced sero-fibrinous exudation without there being any localization of the process within definite portions of the tissue.

SYMPTOMS.

Incubative Period.

This is very variable but symptoms may appear under natural conditions in from three to six days with a minimum of 10 days after contact.

It is possible to distinguish according to the rapidity of evolution three forms, viz.: An acute, a per-acute, and a sub-acute.

Acute form.—In the early stages the symptoms give no precise significance, within the next few days symptoms become more marked. Animal is disinclined to move, skin is dry, hair erect, respiration accelerated, the temperature becomes elevated and may reach as high as 104° Fahr. or over, milk secretion diminishes. The local symptoms now manifest themselves, there is a short dry painful cough which is provoked by percussion of the chest wall.

The symptoms now become intensified, animal is disinclined to move and stands with the head extended and elbows turned outwards, temperature remains elevated; there is a partial anorexia; during inspiration the nostrils are widely opened, the stronger extension of the thorax is followed by a double contraction of the abdominal muscles, the cough becomes more frequent, moist and painful, expiration is accompanied by a groan.

Percussion of the chest wall indicates usually in the lower parts of the affected lung, behind the shoulder and up to a certain height, a partly or entirely dull sound limited above by a horizontal line (pleural exudate) or a zone of dullness and hepatization. Auscultation at first shows a weakened vascular breathing and some crepitation in the anterior and lower parts, later in the region of the partially or entirely dull area no vesicular or bronchial breathing may be heard but this is usually perceptible around the borders of the affected area.

In the later stages, the animal is exhausted, muscular tremors occur. There is usually a muco-purulent discharge from the nostrils, respirations become discordant.

There is often an oedematous infiltration of the dependent parts of the chest and abdominal wall.

Under this form the evolution is complete in from 2-3 weeks. In a percentage of cases the disease comes to a standstill, and the symptoms gradually subside, the cough persists and becomes stronger, respiratory difficulties subside. The appetite, rumination and milk secretion return to the normal, animal gains condition, and after the lapse of a varying period of time the animal although it appears to have recovered, remains a source of infection for a varying period.

In animals kept housed and well fed, the percentage of recoveries is greater than in cattle exposed under adverse field conditions.

Per-acute form.

Exceptionally the disease may have an acute onset with severe symptoms, the lesions develop with a sharp rise of temperature. The symptoms differ according to whether the pleural or pulmonary

lesions predominate; when the pleurisy is intense percussion and auscultation indicate the presence of an abundant exudate; when the pulmonary lesions prevail the cough occurs from the commencement.

Termination by asphyxia is the rule; death occurs in from 2-8 days.

Sub-acute form.

Sometimes the lesions remain localized in a small part of the lung; in such cases, the only symptoms may be a rare cough; percussion and auscultation does not furnish any precise indication. Sometimes new foci of infection occur in the lung and the animal shows acute symptoms, but usually the lesions disappear and the disease escapes detection.

DIAGNOSIS.

In the Living Animal.

Diagnosis particularly of the first case or two of an outbreak, cannot be assured by clinical examination; if the symptoms are suspicious, the history of the movements of the cattle belonging to the herd and the possibility of an infected beast having been introduced should be investigated; serum diagnosis methods could be employed if facilities exist for such, but slaughter of a suspect beast for post-mortem examination and collection of material for pathanatomical and histological examination and cultural tests is desirable.

Pathological-Anatomical and Histological Diagnosis.

The microscopic lesions, viz., exudative pneumonia and pleuritis and sequester formation are as a rule characteristic, yet they may sometimes be confused with other pneumonias, e.g. the pectoral form of haemorrhagic septicaemia of bovines; on the other hand specific histo-pathological changes are usually demonstrable.

Meyer (1909) found that the thick rings of leucocytes with deposits of fibrin which surround the blood vessels of the interstitia in circular symmetrical arrangement can be used with certainty for diagnosis.

Ziegler (1921) concludes that necrosis and the perivascular and marginal organization processes in the interstitia and the parabronchitic changes are specific.

Seifried (1926) concluded that it is necessary to consider separately the histo-pathological changes which occur in the (1) initial and (2) middle stages and (3) the sequel of stages 2 and 3, and that although the perivascular and marginal organization processes of the interstitia are a regular occurrence in the middle stage (acute bovine pleuro-pneumonia) yet they may occur in some other pneumonias; nevertheless the existence of the parabronchitic "Herden" furnish a sure proof of the bovine pleuro-pneumonia nature of doubtful pneumonias. This investigator finds that in the initial stages the parabronchitic and perivascular "Herde" may be wanting and the possibility of confusion with other broncho-pneumonias is then extraordinary great, and that in the sequel and termination of the initial stage the perivascular "Herde" are not to be found in the majority of cases, but the parabronchitic "Herde" can be usually demonstrated in the inner half of the encapsulated portion in which case differential diagnosis is possible.

Cultural tests.—The pulmonary or pleural exudate is diluted in Martin's peptone beef bouillon medium in the proportion of 2 of exudate to 98 of the medium, and 10 per cent. of bovine serum, filtered through the Berkefeld, added, and the tubes incubated at 38° Cent.; if the fluid becomes opalescent in 3 to 4 days and microscopically there is no distinguishable bacterial infection, the diagnosis may be considered as established.

The method introduced by Marino (1905) is of assistance in confirming results of cultural tests.

Biological Tests. The impossibility of detecting by clinical means animals with latent lesions, the so-called "Lungers," and the role which they play in the maintenance and spread of infection prompted workers to determine the value of certain biological tests for diagnostic purposes, viz., agglutination, complement fixation, conglutination reaction and precipitation methods. In addition to the above the lipoid fixation (Meinicke Bley) and the flocculation reaction of Sacks and Georgi, were investigated.

None of the methods are infallible, but the percentage of errors which occur with one may possibly be reduced by the simultaneous application of another.

Their practical value is reduced owing to the necessity of repeating the tests at intervals so as to exclude the risk of animals in the incubative stage of the disease, as well as animals which may have contracted the disease subsequent to the date of the original test, escaping detection.

Should the test fail to reveal all infected animals, and restrictions of movements of the herd be removed, serious consequences are likely to occur particularly in the case of trade and transport cattle which may originate widespread foci of infection.

Agglutination.—Although agglutinins have been demonstrated in the serum of some naturally infected or hyper-vaccinated cattle, and the agglutination method is of some assistance, yet the percentage of errors is too high for it to be used alone, but it could be employed in conjunction with other tests, viz.:—complement fixation or conglutination.

Complement Fixation Method.—This is of some practical value. Titze and Giese (1919) and Titze, Gieze and Wedemann (1923) recorded satisfactory results, but found haemolysis subsequently occurred in the tubes, which showed a fixation, necessitating the reading of the tests within a time limit, this difficulty was however overcome by using culture antigen.

Heslop (1921) and (1922) concluded the method is not sufficiently accurate to warrant its general use, the main difficulty being to prevent errors owing to haemolysis which occurs consequent on the presence of conglutinin and some excess of complement. Dahmen (1922) and (1923) recorded observations on the value of various antigens; Nakamura, Futamara and Watamiki (1926) found with a suitable antigen the complement fixation method reliable.

Walker (1923) obtained practical results and found the haemolysis which occurs in the complement fixation method is eliminated by the use of the conglutination method.

PRECIPITATION METHOD AND ITS MODIFICATION.

Precipitin and precipitinegen have been demonstrated in the serum of infected cattle, and the latter in extracts of hepatized lung. The percentage of errors limit the practical value of the methods.

LIPOID FIXATION (MEINICKE BLEY) AND SACKS AND GEORGI FLOCCULATION REACTIONS.

Titze, Giese and Wedemann (1923) obtained unsatisfactory results and were unable to express an opinion on the practical worth of the methods.

ALLERGIC REACTION.

Siedamgrotsky and Noack (1892), Walter (1892), Beitzen (1919), carried out diagnostic inoculation with sterilized pulmonary serosity and Titze, Giese and Wedemann (1923) with concentrated sterilized culture and obtained temperature reaction in a percentage of infected as well as non-infected cattle. The test is not considered of sufficient practical value to introduce into the practice. Since results depend on a temperature reaction, it would be of less value in tropical countries owing to the variations in temperature which occur in healthy non-housed cattle.

The latter workers found the intrapalpebral and ophthalmic infection of concentrated pure culture of no assistance.

CONTROL MEASURES.

These comprise preventive inoculation, slaughter of affected animals, quarantine and in addition serum diagnosis tests if facilities for such exist.

Preventive inoculation has been made largely use of since Willems (1852) demonstrated that inoculation with pulmonary or pleural sero-fibrinous exudate (lymph virus) confers a resistance against natural infection and re-inoculation.

Pure culture vaccine is now generally substituted for lymph virus where facilities exist for its preparation.

Walker (1921) issued an attenuated pure culture vaccine; in some countries, viz., Great Britain, United States of America and in various European countries preventive inoculation, slaughter of affected animals and quarantine measures were found insufficient and extreme measures, viz., the stamping out method whereby all infected and in contact animals were slaughtered was found necessary to control and eradicate the disease.

South Africa was officially declared free of bovine pleuropneumonia in 1914. The measures adopted there consisted of preventive inoculation, slaughter of visibly affected animals and quarantine of the herd for 3 months.

In the writer's experience a large number of infected cattle recover and are no longer a source of infection, and this fact would appear partially to explain the successful eradication of the disease in South Africa.

In countries in which the disease is widespread generalized periodical preventive inoculation should be of assistance in reducing the incidence of the disease when slaughter of the visibly affected could be considered.

In the case of isolated outbreaks slaughter of infected and in contact animals is usually desirable.

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