OBSERVATIONS ON SOME BIOLOGICAL CHARACTERISTICS OF ORGANISMS OF THE PLEUROPNEUMONIA GROUP[1]

JOEL WARREN*

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York

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Within the past few years there has arisen a renewed interest in that group of bacterial forms designated as the "Pleuropneumonia Group," the prototype of which is *Pleuropneumonia bovum*, first described by Nocard and Roux in 1898, and for many years regarded as a virus because of its filterability. However, the cultivation of this organism on cell-free media, and the later complete description of a similar form as causing agalactia of sheep (Bridre and Donatien, 1925) removed them from this category. Of recent years the group has increased in number with some 20 different pleuropneumonia-like microorganisms being reported from highly varied sources.

The common features which in general separate the pleuropneumonia organisms from the larger bacterial forms and filterable variants of the latter are: (1) A slow rate of growth, optimum for some strains being attained only after 4 to 6 days; (2) they require a high, 10–20 per cent, content of animal protein in the form of serum, etc., in the medium; (3) they stain poorly with the common aniline dyes but are well colored by the polychromatic stains, such as Giemsa; (4) morphologically they may exhibit a complex developmental cycle with asteroid, branching or granular forms being the predominating elements. Although no exact determinations of their size have been made, the ability of these

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* Present address; Childrens Hospital Research Foundation, Cincinnati, Ohio.

forms to pass through Berkefeld V filters would seem to be due to the small size of the elementary bodies, perhaps less than 0.15μ. Numerous authors have evolved highly ingenious, if fruitless, theories postulating "life cycles" but as the latter are so dependent upon the age of the strain, media, means of illumination, etc., we shall not consider them in our discussion but refer the readers to the papers of Klieneberger, Dienes, Ledingham, Nowak, Smiles, Turner and Wroblewski.

Reference will be made to one organism which on first acquaintance seems to be unrelated to the Pleuropneumonia Group, *Streptobacillus moniliformis*. Through the extended work of Klieneberger (1935) a microbe designated by her as the L_1 organism has been found in association with *Streptobacillus moniliformis* which morphologically and in its growth characteristics resembles members of the Pleuropneumonia Group, although no serological relation has been found to exist between L_1 and the rest of the group. A controversy prevails as to whether the L_1 organism represents a variant phase of the *Streptobacillus* (Dienes, Dawson and Hobby) or whether it is a symbiont found in close association with this bacterium (Klieneberger, 1935).

All strains grow aerobically, less profusely in the absence of oxygen. The organism of bovine pleuropneumonia is capable of fermenting glucose, maltose, dextrin, levulose, fructose, mannose, without gas formation. This microbe will reduce hemoglobin and is bile-soluble, (Tang, Wei, McWhirter and Edgar, 1935). Holmes and Pirie (1932) in a more detailed study of the metabolism of *Pleuropneumonia bovum* found that the rate of formation of lactic dehydrogenase paralleled the amount of growth in the culture. Lactic acid was the only hydrogen donor identified, although glucose, formate, succinate, alanine and hypoxanthine had no effect on the capacity of centrifuged cultures to reduce M/1000 methylene blue. There was little deamination of meat broth and no change in the number of free NH_2 groups as measured by the formol titration method.

These results of Holmes and Pirie constitute the sole detailed study of the metabolism of any member of the Pleuropneumonia Group. Accordingly it has been our endeavor to examine the

more recently discovered members of the group with a view towards determining whether similar metabolic principles prevailed for all. As these microorganisms have been somewhat off the beaten path of bacteriology the following short descriptions of the strains used may not be amiss:

1. *Pleuropneumonia bovum* (Nocard, Roux, 1898) (*Asterococcus mycoides, Mycoplasma peripneumoniae, Asterococcus peripneumoniae* (bovis)). The causative agent of pleuropneumonia of cattle, a contagious febrile disease characterized by an exudative, fibrinous pneumonia and pleurisy. Once widespread it is now most prevalent in South Central Africa, Russia and the Balkans.

2. *Organism of Agalactia* (Bridre and Donatien, 1925). A disease almost entirely confined to sheep and goats. It is contagious, with suppurative lesions of the mammary glands, the articulations and the eyes as salient features. Morphologically this form is identical with *Pleuropneumonia bovum* although serologically separate.

3. *Asterococcus canis* (Schoetensack 1934). This organism was isolated in China from 14 of 15 dogs dead of distemper but seems to have no relation to this disease. It closely resembles *Pleuropneumonia bovum* in its morphological structure but is antigenically distinct.

4. *Streptobacillus moniliformis* (Levaditi, Nicolaux, Poincloux, 1925) (*Streptothrix muris* (*ratti*), *Streptothrix putori, Haverhillia multiformis*). This is a gram negative, non-motile, non-capsulated highly pleomorphic bacillus existing as rods, clubs, fusiform or granular forms or any conceivable intermediate. First described by Schottmuller (1914), it has been frequently found to be the incitant of a syndrome in man occasionally following rodent bites and comprising a febrile arthritis, a macular or morbilliform eruption, and lymphadenitis. Spontaneous epizootics of arthritis in mice due to *Streptobacillus moniliformis* have been described by Levaditi, Selbie and Schoen (1932) and by Mackie, van Rooyen and Gilroy (1933). The cumulative work of Tunnicliff, (1916), Strangeways (1933), and Klieneberger and Steabben (1937) demonstrated that this organism is a common inhabitant of the nasopharynx and bronchial tree of the rat.

5. L_1 *Organism* (Klieniberger, 1933). The term "L Forms" was originated by Klieneberger (but with no published explanation of their significance). When a culture of the Streptobacillus was plated out on an enriched ascitic fluid agar there developed in association with it a number of extremely minute colonies quite unlike those of the original

inoculum. These were designated as "L_1 Organisms." When isolated and subcultured they appeared to be of a characteristic morphology and colonial structure. With the exception of one strain, the "L_1 old," the organisms when cultivated contain both the L_1 and Streptobacillus elements. Because of this and their serological crossing with the respective antisera they are regarded by myself as variant forms of the same microorganism. In our hands L_1 has not been pathogenic for any laboratory animal.

6. *L_3 Organism* (Klieneberger, 1938). A serologically individual type first isolated from a lung lesion in a "tame rat." Pathogenic for mice after intraperitoneal inoculation with the formation of abscesses.

7. *L_4 Organism* (Klieneberger, 1938). This pleuropneumonia-like form, of murine origin, when first isolated exhibits a marked pathogenicity for the rat and mouse, producing multiple pyogenic abscesses in various organs and a high frequency of pyoarthroses. It is antigenically distinct from other members of the group.

8. *L_5 Organism, "Strain A" of Sabin* (Sabin 1938, Findlay, Klieneberger, MacCallum and Mackenzie, 1938). Sabin, while studying the nature of a neurolytic factor in *Toxoplasma*-infected mouse brain, cultivated a pleuropneumonia-like form which is distinctive in its ability to produce an exotoxin having a striking affinity for the cerebellum of the mouse. Simultaneously Findlay and his associates isolated the L_5 organism from mice developing central nervous signs after being inoculated with the virus of lymphocytic choriomeningitis. Cross agglutination experiments indicate that L_5 and the "A" strain are identical.

METHOD

All strains were maintained in phosphate infusion broth, pH 7.8, containing 20 per cent of horse serum. Transfers, by pipette, were made every 48 hours except in the case of the L_4 organism which grows quite slowly and is subcultured at 4-day intervals. When plate cultures were used it was found that a moist chamber prevents the drying out of the agar over prolonged periods of incubation. An anaerobic jar containing a cotton pad moistened with 1:100,000 $CuSO_4$ solution serves admirably and decreases the occurrence of molds. The presence of growth upon solid media should always be determined by microscopic examination as the colonies are small and often sparse.

For the examination of individual organisms either the dark-field or Giemsa stained films was resorted to.

The following strains were very kindly supplied by the workers mentioned and used throughout the study:

Streptobacillus moniliformis (Dienes). Obtained from Dr. L. Dienes who isolated it from the lung lesion of a white rat.

Streptobacillus moniliformis (Cook). Obtained from Dr. M. H. Dawson who isolated this strain from a case of rat-bite fever.

Streptobacillus moniliformis (Crocker). From the nasopharynx of an old rat suffering from "snuffles."

L_1 (Dienes). Isolated by Dienes from his strain of Streptobacillus.

L_1 (Cook). Isolated by Dawson and Hobby from the "Cook" strain of *Streptobacillus.*

L_1 "old". From Dr. Klieneberger; this strain has never reverted to the *Streptobacillus.*

L_3 (Dienes). From a lung lesion in a laboratory rat. From Dr. Dienes.

L_3-5254. From Dr. Klieneberger.

L_4-72 "gland." From Dr. Klieneberger.

L_4 "V." Obtained by Woglom and Warren (1938) from an infected rat sarcoma.

L_4 "K." Isolated by the author from the brain of a mouse experimentally infected with herpes febrilis virus.

L_5. Supplied by Dr. Klieneberger.

Asterococcus canis, Type 1. Received from Dr. Klieneberger.

Pleuropneumonia bovum. From National Collection of Type Cultures No. 4159.

Pleuropneumonia bovum. From National Collection of Type Cultures No. 3278.

Pleuropneumonia bovum. From National Collection of Type Cultures No. 4732.

Organism of *Agalactia.* From National Collection of Type Cultures No. 3722.†

EXPERIMENTAL

1. The growth of Pleuropneumonia bovum and pleuropneumonia-like forms in increased concentrations of carbon dioxide. Thirty per cent horse serum agar plates were inoculated with 0.1 ml of

† Examination of films of this strain of Agalactia show it to be a small cocco-bacillus, morphologically unlike other members of the Pleuropneumonia Group. As we were unable to test this strain serologically it is quite possible that it is a contaminant and the results indicated for "agalactia" should be regarded with caution.

broth culture and placed in jars containing air-CO_2 mixtures of varying ratios. To the agar was also added 1 per cent of phosphate buffer, pH 7.8, to prevent too rapid changes in the pH of the medium. The plates were examined daily for the presence of growth.

The growth of *Streptobacillus moniliformis* and the L_1 organism was markedly enhanced by the increase of CO_2 to 10 per cent, large, confluent colonies appearing within 24 to 48 hours (table 1). Above this concentration there was a striking depression in the number of colonies and at 30 per cent there was no growth on any of the plates. The L forms and *Asterococcus canis* on the other hand grew but sparsely at a concentration of 10 per cent CO_2

TABLE 1

The effect of increased CO_2 concentration on growth

CULTURE	10 PER CENT CO_2	20 PER CENT CO_2	30 PER CENT CO_2
S. moniliformis	Growth	Growth	No growth
L_1	Growth	Growth	No growth
L_3	Growth	No growth	No growth
L_4	Growth	No growth	No growth
L_5	Growth	No growth	No growth
Asterococcus canis	Growth	No growth	No growth
Agalactia	No growth	No growth	No growth
Pleuropneumonia	No growth	No growth	No growth

while the multiplication of the *Agalactia* and *Pleuropneumonia bovum* strains was completely inhibited at this concentration.

It was noted by Levaditi (1925) that the evacuation of O_2 from the culture vessel containing cultures of *Streptobacillus moniliformis* was attended with better growth. This was probably due to an increase in the proportion of CO_2 in the vessel rather than decreased O_2 tension as in our experience anaerobic and semi-anaerobic environments are not very favorable for any of these strains although all will grow to some extent anaerobically.

2. Fermentation of carbohydrates. In view of the fact that the sugar fermentations of *Streptobacillus moniliformis, Pleuropneumonia bovum,* and *Agalactia* have been previously determined it was hoped that a comparison of similar reactions on the part of

the L organisms might afford a biological basis for their classification.

Washed cultures of these microorganisms did not produce significant pH changes in solutions of various carbohydrates. Hence the strains were cultivated in a fluid medium of 20 per cent horse serum, 3 per cent broth, 75 per cent water and 2 per cent of the carbohydrates.

Experiment 1: The diluted broth was inoculated with 0.1 ml. of a 48-hour culture, the tubes incubated for 4 days and daily pH values obtained. Uninoculated tubes of medium and sugar were observed in a similar manner as controls. A difference in pH of 1.0 or more between the inoculated and uninoculated tubes was considered as significant acid production.

TABLE 2

Carbohydrate fermentations

ORGANISM	LACTOSE	GLUCOSE	MALTOSE	MANNITOL	INULIN	SALICIN	SUCROSE
S. moniliformis....	Acid	Acid	None	None	None	None	None
L_1.....	Sl. acid	Acid	None	None	None	None	None
L_3.....	None	Acid	None	None	None	None	None
L_4.....	None	Acid	None	None	None	None	None
L_5.....	None	Acid	None	None	None	None	None
Agalactia.....	Sl. acid	Acid	Acid	Variable	None	None	Acid
Pleuropneumonia..	Acid	Acid	Acid	Acid	None	None	None

Gas was never produced. It will be noted from table 2 that only the *Pleuropneumonia* and *Agalactia* strains will split maltose and mannitol, while glucose is fermented by all members of the *Pleuropneumonia* group. The end determinations of pH were of interest as *Streptobacillus moniliformis* and the L_1 organism produced greater acidity than any of the other L forms. After four days of cultivation values for these two organisms were in the region of pH 4.8–5.0. We had previously noticed that cultivation of *Streptobacillus moniliformis* in glucose broth resulted in death of the culture in 48 hours. That this was probably due to the acid produced was proved by adding N/20 HCl to cultures in plain broth. When a pH of 4.8 is reached viable subcultures cannot be obtained.

The strains of L_3, L_4 and L_5 only attacked glucose, final values of pH being in the neighborhood of 5.6–5.8. In the case of *Pleuropneumonia bovum*, Holmes and Pirie found that the splitting of carbohydrate determined its R.Q. for the first 48 hours of growth. Is this carbohydrate the main energy source of these forms or are other substrates the major source? This question was attacked in the following manner.

Four-day-old serum broth cultures of the L strains and *Asterococcus canis* were centrifuged for one hour at 3200 r.p.m., the sediment washed twice with M/15 phosphate buffer and resuspended in phosphate to one twentieth the original volume. Portions of this material were then mixed in separate tubes with

TABLE 3

Reduction of methylene blue in presence of specific substrates

ORGANISM	BROTH	SERUM	LACTATE	FORMATE	SUCCINATE	PYRUVATE	HYPOXANTHINE	CYSTINE
L_1	++++	++++	++	0	0	0	0	0
L_2	++++	++++	++	0	0	+	0	0
L_4	++++	++++	++	0	0	0	0	0
L_5	++++	++++	++	0	0	0	0	0
Asterococcus canis	++++	++++	++++	0	0	0	0	0

++++ Dye completely decolorized in 12 hours.
++ Dye partially decolorized in 12 hours.
0 Dye not decolorized in 12 hours.

1.0 ml. of the following substrates: broth, horse serum, 1 per cent sodium lactate, 1 per cent sodium formate, 1 per cent sodium succinate, 1 per cent sodium pyruvate, 0.1 per cent hypoxanthine, 0.1 per cent cystine. 0.1 ml. of thousandth molar methylene blue was then added, the tubes sealed and incubated at 37 degrees. Uninoculated tubes of the substrates were used as controls. Continuous observations were made of the amount of reduction of the dye. See table 3.

All of the L forms tested, whether of rat or mouse origin, behave toward these substrates in a similar manner. Only broth, serum and to a lesser degree sodium lactate acted as hydrogen donors. It was noted that the strains of L_3 were capable of

reducing the methylene blue much faster in serum or the phosphate broth than any of the other microorganisms.

Thus, there is little difference in the oxidative activity of these forms upon certain carbohydrate substrates when the above method is utilized. Whether or not these microorganisms as well as the *Pleuropneumonia bovum* strains are also limited in their capacity for deamination and proteolysis awaits further work. There is a close similarity existing between members of the group and it is quite possible that a common metabolic scheme prevails for all of these microorganisms. Their inability to perform many of the basic hydrogen transfers will perhaps explain their slow and often precarious growth in highly nutritive media.

3. Some observations upon the oxidation-reduction potentials in cultures of members of the pleuropneumonia group. Using the platinum electrode we have made a series of measurements of the oxidation-reduction curves of our strains over their entire growth period. The technique was the same as employed by Warren, Street and Stokinger for the streptococcus. All cultures were grown aerobically in 20 per cent horse serum phosphate broth of pH 7.8.

Typical time-potential curves are shown in chart 1. Cultures of *Pleuropneumonia bovum* and *Agalactia* have similar, and in comparison with the other Pleuropneumonia-like strains, characteristic Eh curves. From the time of inoculation until the 22nd hour there is a gradual drop in potential. In the region of -0.20 volts there is no further reduction and the voltage remains stationary. These curves are of interest when we consider the findings of Holmes and Pirie concerning the fermentation of glucose by *Pleuropneumonia bovum.* In their experiments about 90 per cent of the added glucose was broken down on the second day of the culture. With our strains, since the entire drop in potential occurred in the first 24 hours of growth, it would appear that some mechanism other than glucose fermentation was primarily responsible for this reduction.

None of the other Pleuropneumonia-like forms gave time-potential curves resembling the above. The L_3, L_4, and L_5 strains caused a very slight but prolonged drop in voltage. Test platings

on all of these cultures gave considerable growth although it was not reflected in a large drop of Eh. However, it must be borne in mind that the presence in media of large amounts of serum tends to keep the potential elevated (Hewitt, 1932). From these curves it will be seen that *Pleuropneumonia bovum* and *Agalactia*

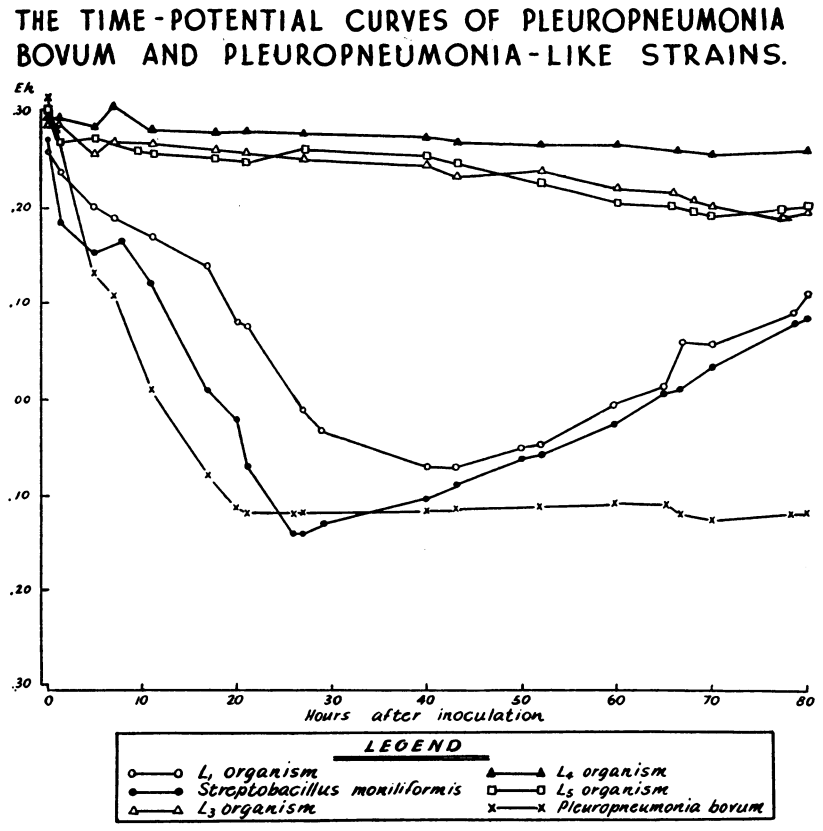

CHART 1

on the one hand, and L_3, L_4, and L_5 on the other behave as entirely dissimilar microorganisms under the conditions of the experiment. Furthermore, these L forms are capable of proliferation at somewhat elevated Eh levels as compared to most of the larger bacterial species.

The oxidation-reduction curves of *Streptobacillus moniliformis*

and the L_1 organism are alike. The medium is actively reduced by about 0.400 volt in the first 20–24 hours, after which there is a gradual rise in potential until the third day where it is maintained in the region of Eh + 0.060–0.120 volt. This close similarity between these morphologically dissimilar organisms serves to increase the importance of the problem of whether they are variants or symbionts as they appear to be metabolically alike insofar as their rate of reduction of a broth medium is concerned.

4. The relation between virulence and methylene blue reduction time. In our experience one of the striking properties of the L_4 organism in particular is its rapid loss of virulence when grown in liquid media. Three passages in serum broth is sufficient to abolish almost completely its infectivity for the rat, although not for the mouse. After ten passages the "V" strain will no longer produce lesions in either animal. It was found that anaerobic cultivation of L_4 will preserve its virulence for at least eight subcultures. We were curious to determine therefore, whether the virulence of this strain was associated with the preservation of reducing activity. As we have cited, Levaditi had previously found that *Streptobacillus moniliformis* also retains its pathogenicity better if grown in the absence of O_2 and this organism was also included with L_4 in these experiments. Unfortunately, our L_1 and L_3 strains were avirulent.

Broth cultures were centrifuged and the sediments resuspended in one-tenth the original volume of broth. The cultures used had been cultivated in the broth medium for a varying number of four-day passages, all initial inoculations into liquid media having been made with pus obtained from the brain lesions of intracerebrally infected mice. Two milliliters of each suspension was mixed with 0.1 ml. of M/1000 methylene blue and the tubes sealed and incubated. Control tubes contained broth alone and the indicator. Mice were also inoculated intravenously with 0.3 ml. of each subculture in order to determine their relative virulence. In the table the fractions indicate the number of animals showing symptoms out of the total infected. Table 4 indicates the amount of methylene blue reduced by cultures which had been in artificial media for increasing lengths of time.

It will be observed that the cultivation of these two microorganisms on artificial media is attended with a gradual decrease in their ability to reduce methylene blue in the presence of broth.

Parallel with this the virulence of each successive subculture gradually diminished so that the last culture tested produced no demonstrable lesions in mice. While these experiments were performed with only two types of microorganism they suggest that the labile pathogenicity of some members of the pleuropneumonia-like group is associated with a loss of dehydrogenase activity.

A similar disappearance of reductases has been found by MacLeod (1939) to occur in pneumococci which have become sulfa-

TABLE 4

Relation between virulence and methylene blue reduction time

	30 MINUTES	60 MINUTES	24 HOURS	VIRULENCE
S. moniliformis:				
First subculture	++	+++	++++	3/3
Third subculture	++	+++	++++	3/3
Sixteenth subculture	−−	+	++++	1/5
L_4 organism:				
First subculture	−−	+	++++	3/3
Third subculture	−−	−−	++	2/3
Fourteenth subculture	−−	−−	+	0/5

pyridine-fast, although in this case their virulence was unimpaired.

5. Hemolysin production. Berkefeld "N" filtrates of L_1, L_3, L_4 and L_5 organisms and *Agalactia* are unable to hemolyse suspensions of washed rabbit erythrocytes. When cultivated on 10 per cent bovine serum agar with 0.5 per cent of added sheep blood the L_3 organism occasionally produces a green discoloration of the plate similar in all respects to that occurring with *Streptococcus viridans* but limited to those portions of the plate covered by the colonies with no diffusion beyond this area. As has been previously demonstrated by Tang, Wei, McWhirter and Edgar (1935), *Pleuropneumonia bovum* will reduce hemoglobin either in solid or fluid media. This property was not observed with any of our other Pleuropneumonia-like strains.

6. Production of an exotoxin. Since the "A" strain of microorganisms (L_5) discovered by Sabin, and by Findlay *et al.* produces a readily demonstrable exotoxin, it was deemed advisable to determine whether any of the other members of the Pleuropneumonia group possessed this property. Accordingly, 0.3 ml. of the Seitz filtrates of 96-hour serum broth cultures was inoculated intravenously into 4-week-old mice but with no symptoms resulting when the following strains were used: L_1, L_3, L_4, *Agalactia, Pleuropneumonia bovum* and *Streptobacillus moniliformis.* With filtrates of the latter organism an occasional mouse will die within 24 hours following the injection, but at autopsy there is neither gross nor microscopic pathology.

7. The effect of ultraviolet irradiation upon pleuropneumonia-like microorganisms. Four-day-old agar plate cultures of the following strains were exposed for one hour to a mercury vapor lamp at a distance of 24 centimeters; *Streptobacillus moniliformis,* L_1, L_3, L_4, L_5, *Asterococcus canis, Agalactia,* and *Pleuropneumonia bovum.* Small blocks of agar were then cut out from each plate and cultured in 10 per cent serum broth. It was found that all of these microbes will withstand one to two hours of exposure but are killed after three hours.

8. Resistance to hydrogen peroxide and phenol. Because viruses in general are sensitive to the lethal action of hydrogen peroxide, the question arises whether these microorganisms, of a size commensurate with the larger viruses, would also prove susceptible to inactivation by small amounts of this oxidant while fairly resistant to phenol as found by Woglom and Warren (1938) to hold true for L_4.

Our procedure consisted of a determination of the amount of peroxide which would destroy a 72-hour serum broth culture of the organisms after 24 hours of contact at 37 degrees. The final results are presented in table 5.

These concentrations of peroxide are not unlike those required to kill other aerobic bacteria, such as the streptococcus and the pneumococcus, as reported by McLeod and Gordon (1923). They are however higher than those lethal to some viruses, as poliomyelitis.

It should be borne in mind that a technique involving suspen-

sions of organisms in fluid media has a source of error in the catalase present in the serum broth. Also, the variation in the number of organisms per milliliter in different strains makes the relative resistance of these strains of small significance. In general, the members of the Pleuropneumonia group appear to have a similar peroxide sensitivity, which is not unlike that of the larger bacterial species.

Resistance to phenol was measured in a manner similar to that used for peroxide except that the mixtures of phenol and culture were kept in contact for one hour at the end of which time samples were plated on serum agar plates. A quantitative estimation of the actual percentage surviving would be desirable but this is difficult owing to the small size and the fusion of individual

TABLE 5

ORGANISM	LETHAL CONCENTRATION OF PEROXIDE
S. moniliformis	1:20,000
L_1 "old"	1:20,000
L_3	1:10,000
L_4	1:10,000–1:15,000
L_5	1:10,000
Asterococcus canis	1:10,000
Agalactia	1:2500
Pleuropneumonia bovum	1:10,000

colonies upon the plate. For this reason only the survival of a strain in the presence of phenol for an arbitrary period of time is given. By this method it was found that all our forms behaved in a similar manner. A final concentration of 1:200 was lethal, whereas 1:400 had no effect upon the growth of the microorganisms.

9. Resistance to dessiccation. All of the "L" forms, *Asterococcus canis*, and the *Agalactia* and *Pleuropneumonia* organisms can be retained in the dessicated state for at least 62 days. Ten per cent serum broth cultures were concentrated to 1/10 volume on the horizontal centrifuge and dessicated in a Flosdorf-Mudd lyophile apparatus after preliminary rapid freezing at −80 degrees C.

A virulent strain of the L_4 organism was not infective for mice after 40 days of dessication. However, the strain was easily recovered from the dried state indicating that although it had lost its virulence, it was nonetheless viable.

DISCUSSION AND CONCLUSION

Our original intention has been to investigate some of the biological characteristics of the more recently discovered filterable microorganisms which in their morphology resemble the causative agent of bovine pleuropneumonia. We find that in general these "L" forms as well as *Asterococcus canis* have similar properties. Only glucose of the carbohydrates tested, was fermented and this sugar to an equal degree by all strains. A dehydrogenase for lactate was present in all forms and this again was the only one found in the group tested. Considering the little activity which these strains manifest on carbohydrates as well as their high serum requirements, a study of their proteolytic and deamination processes would be highly informative.

The *Streptobacillus moniliformis* and the L_1 organism show a close similarity in their oxidation-reduction curves and fermentations, but with the exception of this finding there are no marked differences between L_1 and any of the other strains so that we cannot from the findings herein reported venture any definite opinion as to whether the L_1 is a *S. moniliformis* variant or a distinct but symbiotic bacterium. Not enough is known concerning the Eh potentials in either bacterial variants or symbionts to assess the importance of the closely similar curves obtained for these two microorganisms.

In view of the finding that *Pleuropneumonia bovum* and *Agalactia* strains have different time-potential curves from the "L" forms it appears that the latter, all rat or mouse strains, have related potentiating systems. In one experiment, the potentials of a strain of L_4 which had undergone repeated subculture were compared with those of one freshly isolated from the rat. There was no difference in the shape of the curves although the voltage dropped more slowly in the stock culture. This result is what we had anticipated from the methylene blue reduction experiment with these two cultures.

The three strains of *Pleuropneumonia* have invariably produced discoloration of the blood pigments when grown in blood broth. This never occurs with any of the "L" forms and will serve as a differentiating characteristic.

It would be highly desirable to obtain further information regarding the nature of the L_5 toxin, a problem upon which our investigation has shed no light. The L_5 organism does not differ in any manner from the other L forms in any of the characteristics described in the preceding.

SUMMARY

1. Eight types of microorganisms at present designated as members of the Pleuropneumonia Group were compared with respect to certain metabolic characteristics.

2. All strains ferment glucose and contain a lactic dehydrogenase.

3. The strains fall into one of three types of time-potential curve, *Streptobacillus moniliformis* and the L_1 organism belonging to one type, the remaining L forms to a second, and *Pleuropneumonia bovum* and *Agalactia* to a third.

4. Associated with the comparatively rapid loss of virulence of *Streptobacillus moniliformis* and the L_4 organism is the disappearance of a reductase for some component of serum broth.

5. Only *Pleuropneumonia bovum* produces a hemolysin.

6. No evidence for the production of an exotoxin appeared when filtrates of cultures of L_1, L_3, L_4, *Agalactia*, *Pleuropneumonia bovum* and *Streptobacillus moniliformis* were injected into mice.

7. In their hydrogen peroxide, phenol and ultraviolet light sensitivity the members of the Pleuropneumonia Group resemble the larger bacterial species.

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