CONTRIBUTIONS TO BIOLOGICAL STUDIES ON THE VIRUS OF CONTAGIOUS PLEURO- PNEUMONIA IN CATTLE

Dr. NORICHICA NAKAMURA, HIROJIRO FUTAMURA and TOSHIO WATANUKI

*(From the Veterinary Laboratory, Ministry of Agriculture
and Forestry, Nishigahara, Tokyo)*

(Received for Publication August 16, 1926)

INTRODUCTION.

For effective control of contagious pleuro-pneumonia in cattle, it is believed of importance to ascertain the biological characters of the virus of the disease. Extensive studies for this purpose have been made abroad, so that there is a number of valuable informations relating to cultural and biochemical surveys. However, biological characteristics of the virus have not been yet thoroughly demonstrated. As shown in our former publication, in growing the virus on culture media some particular efforts should be made to obtain positive results. Moreover, as for the tenacity of the virus against physical and chemical influences, our knowledge seems to be almost lacking. The possibility of the cultivation of the virus in vivo by sealing broth inoculated with a small quantity of lymph from affected lungs in a collodion sack and placing it in the peritoneal cavity of a rabbit was first demonstrated by Nocard and Roux (1898). Later they succeeded in cultivating the virus in the peritoneal cavity of cattle by a similar procedure, and also in Martin's broth added with cattle or rabbit blood serum (6-8 %). Dujardin-Beaumetz found Martin's peptone and Martin's broth agar containing blood serum to be suitable media for the development of the virus outside the animal body. Furthermore, Marino used broth added with a few drops of an alcoholic solution of eosin blue for the estimation of development of the virus in fluid media. Besides these investigators Bordet, Schmit, Poppe (1913), Giese (1922), Joseph, Heslop, and Dahmen published results of their cultural or biochemical researches.

The sudden outbreak of this disease in our country in May, 1925, led us to undertake a more systematic study concerning the biological characters of the virus of contagious pleuro-pneumonia by which various unknown points regarding the cultural and bio-

chemical characters became clearer.

Cultural method.

Five strains of the virus employed in the present investigation are following:

K strain. Received from Mr. Ono, Yokohama, and isolated from the material of an affected Mongolian heifer.

Y strain. Isolated from pleural exudate of an affected cow on the O dairy farm near Osaka. (28/V, 1925.)

N strain. Isolated from a case of the disease on the U dairy farm in Shiga Prefecture. (11/VI, 1925.)

H strain. Isolated from a case of the disease on the S dairy farm near Osaka. (4/VII, 1925.)

D strain. Isolated from the "Lungenseuche-Vakzin" made in Farbwerke vorm. Meister Lucius & Brüning, Germany. (VI, 1925.)

For the cultivation of the virus it is of great importance to determine whether the presence of oxygen will be definitely necessary for the growth of the virus. As regards the influence of oxygen for the development of the virus in vitro, Dahmen demonstrated the necessity of its presence. On the contrary, Ono reported the growth of the virus in vitro under the anaerobic circumstances. So, we carried on cultural experiments in an anaerobic condition complying with Buchner's or Lentz's method. By Buchner's method no growth of the virus was given within first 7 days; but 10-14 days later, a few slightly raised faint colonies, which increased no more in size, as those on a slant agar without blood serum; or turbidity of condensed water was seen. By Lentz's method the growth did not appear until 14th-21st days. There was a capability of aerobic development of the virus grown anaerobically by above methods, though it gave positive results until the sixth or seventh day of anaerobic growth; but no more growth after the eighth day. These experiments showed that in the total absence of oxygen it grows only with difficulty as Dahmen already declared. In shake culture, there develop, at a fixed distance from the surface, numerous similar colonies closely situated just as the growth of *Bacillus abortus* (Bang).

At the same time our experiments demonstrated another condi-

tion for the favourable development of the virus. As is known, the virus has a tendency to form a colony slowly even on a suitable culture medium. As a condition unfit for the growth of the virus may happen in media, a number of culture tubes was closed with cotton as usual, while the other with rubber stoppers, and both were kept in an incubator at about 38° C. for 3-4 weeks. As a result of the two series of experiments, a noticeable difference between both was observed. In the former series the development was not so good: in serum broth turbidity was noticed, and on serum agar there appeared very characteristic delicate colonies, becoming later almost transparent.

In the latter series the growth was much better: fluid media were uniformly clouded within 3 or 4 days, and on solid media there was the formation of marked colonies, surrounded later by a thick peripheral zone. This resulted from necessary moisture which was maintained by tight closing of culture tubes. This technic is also available in preventing the contamination of various bacteria. So in our biological researches cultures were usually made according to this condition.

Preliminary experiments.

There is a known instance in which the addition of normal blood serum to the media used for cultivation of the virus has been claimed to be necessary. Dujardin-Beaumetz considered 10 to 15 % blood serum to be a suitable quantity of the serum and observed no difference between blood sera from the man, horse, cattle, swine, goat, sheep, rabbit, guinea-pig or fowl; but no growth in fluid and coagulated serum. Schmidt observed good development in Löffler's broth containing 25% cattle blood serum. Also, Joseph preferred the cattle blood serum to horse serum (8 %). Giese consistently claimed that alkaline media were the best for the growth of the virus and special efforts have been made to secure reactions of culture media ranging from 7.8 to 8.0 in hydrogen ion concentration.

Now, tests were made to determine the minimum amount of blood serum added to media that could be safely used for cultivation of the virus. For this purpose plain agar (3 %) and broth

added with varying quantities of horse blood serum as shown in following table, were used, the pH being 7.5. Cultures were kept at the same temperature for 3 weeks.

Days for Cultivation	2		3		5		7		10		14		21		28	
Medium	A		B		A		B		A		B		A		B	
Quantity of blood serum	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0	-	-	-	-	-	-	±	-	±	-	±	-	±	-	-	-
0.5 %	-	-	-	-	±	-	+	-	+	-	+	-	±	-	-	-
1 %	-	-	-	-	+	-	+	-	+	-	±	-	±	±	-	±
2 %	-	-	±	-	+	-	±	-	±	±	±	±	±	±	-	±
3 %	-	-	+	-	+	±	±	+	±	±	±	±	±	±	-	±
5 %	-	-	+	-	±	+	±	±	±	±	±	±	±	±	-	±
10 %	±	±	+	+	±	±	±	±	±	±	±	±	±	±	-	±
15 %	±	-	+	-	±	-	±	-	±	-	±	-	±	-	-	-
20 %	±	±	+	+	±	±	±	±	±	±	±	±	±	±	-	±
30 %	-	±	+	+	±	±	±	±	±	±	±	±	±	±	-	±
50 %	-	-	±	-	+	+	+	±	±	±	±	±	±	±	-	±
60 %	-	-	-	-	-	-	-	-	-	-	±	-	±	-	+	+
70 %	-	-	-	-	-	-	-	-	-	-	±	-	±	-	±	±
80 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A agar; B broth; ± Luxuriant growth; ± Good growth;

± Normal growth; + Slight growth; ± Obvious; - No growth.

A series of cultural experiments was performed to determine any difference in the growth of the virus between broth prepared from several kinds of meat as beef, pork, horse, or rabbit flesh. Moreover, on the other hand, several kinds of serum (10%) were added to the media, having a pH of 7.5. Cultural results showed, as a rule, no remarkable difference between them; but luxuriant growth in media prepared from pork broth.

As to solid media, it was found that 10 to 15 per cent was the sufficient amount of blood serum to be added. When the

serum content was less than 5 or more than 20 per cent, the growth was not so pronounced as in the cases of 10 to 15 per cent. As to broth, 20–30 per cent was the most suitable amount, The blood serum, fresh or treated below 70° C., is much better than that heated at a higher temperature. The growth in plain broth was, as a rule, hardly observed. Accordingly such media are not suitable for the cultivation of the virus in practice.

Now, a series of experiments was carried on to determine the limit of hydrogen ion concentration at which the virus of this disease will grow normally. 10 per cent serum agar (3 %) with varying degrees of hydrogen ion concentration was used, the pH ranging from 6.0 to 9, while in 10 per cent serum broth from 5.5 to 9.0. It was found that in both media the virus grew luxuriantly at a pH of 7.5; the growth being as heavy as that in media With more alkaline reaction; the growth at a pH of 7.0 was almost as heavy as that at a pH of 7.6, and there was a only slight growth in media with pH of 6.0–6.5, no more growth at pH of 5.5 or 9.0.

Days of Cultivation	2		3		5		7		10		14		21	
Medium pH	A	B	A	B	A	B	A	B	A	B	A	B	A	B
5.5		—		—		—		—		—		—		—
6.0	—	—	—	—	—	—	±	±	+	+	+	+	+	+
6.5	—	—	—	+	+	+	+	+	+	+	+	+	+	+
7.0	±	±	+	+	+	+	+	+	+	+	+	+	+	+
7.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.5	—	—	—	—	±	—	+	+	+	+	+	+	+	+
9.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—

A agar, B broth.

As to the temperature at which cultures will be kept, the optimum temperature is 36° to 38° C., the growth ceases below 27° C.

Growth on various culture media.

1. Serum agar.

The surface colonies did not appear until the fifth or seventh day, while the condensed water became more or less cloudy, showing a dim growth. The small delicate and almost transparent colonies resembling dew drops were found later, increasing in size so as to be demonstrable macroscopically (their diameters measuring from 0.1–1.0 mm.). The colonies were usually isolated from one another, with a regular outline. Later they showed a slightly thick part in the centre of colonies (frequently two or more); later the part became brownish and adhered firmly to the medium, while the peripheral area was grayish-white. In general, the first generation of culture showed a very slow growth, and the colonies used to develop on the surface adjacent to the condensed water. However, later generations showed a tendency to form an easy growth on the whole surface of the agar within a week; definite colonies, however, were outstanding and characteristic. Moreover, when incubated for a long time, macroscopical changes of colonies were often observed in shape as seen in the illustration. Also the virus was cultivated on the surface of plain agar moistened with drops of serum, as shown by the experiments of Dujardin-Beaumetz.

Shake culture: At 5–10 mm. under the surface, numerous similar colonies developed, closely situated, forming a zone of growth with the breadth of 3 to 5 mm. Later the colonies grew uniformly in the medium.

Stab culture: After 7 days the white cloudy growth developed along the line of stab 3–5 mm. under the surface and later formed a continuous white streak.

2. Amnion or serum gelatine agar.

Shake culture in this medium gave almost the same results as in serum agar culture, but the zone of growth most marked in amnion gelatine agar.

3. Serum broth.

The culture was noticeable by a very delicate turbidity of the fluid and in the course of time a moderately heavy growth was shown, but there was no sedimentation of the growth even after two months, much better growth in serum added with glucose,

saccharose or lactose (1%) broth.

4. Serum peptone-water agar (Serum Martin's peptone-water agar)

In these media the growth took place within 10 days, but the development was not good, so that colonies were more delicate than those on the serum agar. The addition of one per cent glucose, lactose or saccharose to this medium caused usually better growth. As for the limit of concentration of peptone at which the virus will grow normally, a series of experiments was performed and 10 per cent serum peptone-water agar (3 %) of various concentrations was used, ranging from 0.1 to 20 per cent. It was found that the growth was as heavy as that in serum agar; the growth at 20 per cent peptone was almost as impossible as in plain agar.

5. Five per cent egg-yolk agar.

Good growth was obtained within a few days as in serum agar. However, it was not easy to examine the structure of colonies by the opacity of the media. On the contrary, a slight or no growth was obtained on 5-20% egg-white agar.

6. Serum milk.

No change in appearance. It was observed by cultural tests that the virus seems to develop in the medium after about five days.

7. Potato blood serum.

The growth was not good.

8. Bile medium.

The addition of 5-10% cattle bile both to plain agar and broth did not favour the growth of the virus.

Fermentation tests.

0.5 per cent of the following 16 test substances being used, serum litmus peptone water (10 per cent blood serum, 1 per cent peptone water) was inoculated for fermentative tests.

Pentoses: xylose and arabinose.

Hexoses: glucose, laevulose, galactose and mannose.

Monosaccharides: mannite and isodulcite.

Disaccharides: lactose, maltose and saccharose.

Polysaccharides : soluble starch, dextrin and inulin.

Glucosides : salicin.

Monoamino-dicarboxylic acid : asparagine.

A set of uninoculated tubes was used for control.

The growth of the virus in these media being examined by cultural test at the end of three weeks, the fermentative reaction was determined in colour comparing with the control tube. As seen in the following table, eight of test substances were fermented with acid production in the course of five or ten days. However, in the case of laevulose, xylose and arabinose the acidity was less than in the case of the five sugars, namely, glucose, mannose, maltose, dextrin and soluble starch. In other cases any colour change was not observed at all.¹ In testing acid production, our experiments showed serum fuchsin agar with 0.5 per cent glucose, mannose or maltose to be suitable for the purpose, reddening of the medium being very pronounced within 5 or 7 days.

Days of Cult. Subst. Tested	3	5	7	10	14	21
Glucose	+	+++	+++	+++	+++	+++
Mannose	+	+++	+++	+++	+++	+++
Galactose	-	-	-	-	-	-
Laevulose	-	-	±	±	-	-
Lactose	-	-	-	±	±	±
Maltose	+	+	+++	+++	+++	+++
Saccharose	-	-	-	-	-	-
Dextrin	+	+++	+++	+++	+++	+++
Mannite	-	-	-	-	-	-
Xyrose	-	-	-	±	+	+
Arabinose	-	-	-	±	+	+
Asparagine	-	-	-	-	-	-
Inulin	-	-	-	-	-	-

Salicin	-	-	-	-	-	-
Isodulcite	-	-	-	-	-	-
Starch	+	+++	+++	+++	+++	+++

Besides this, the formation of indol and the occurrence of SH_2 were not demonstrated even after a long incubation. Also, the haemolytic action against blood corpuscles of several animals was not recognized.

Resistances against the physical and chemical influences.

According to Nocard, Roux and Beaumetz, the virus closed in glass tubes and kept at a temperature not exceeding 12°C ., retained the virulence for almost one year, while cultures kept in the incubator and transferred every two weeks retained their original virulence not longer than 6 to 8 days. Laquerriere's observations showed that frozen pieces of hepatized lung kept at a temperature of 5° to 6°C . contained virulent lymph even after three months, while a temperature of 58°C . destroyed the virus. Schütz and Steffen, and Nocard described that the virulence was not influenced by concentrated glycerine or by 0.5% carbolic acid and the latter recommended carbolic acid in 1:2000 for preservation of the virus. However, the present knowledge is not enough for device of practical destruction of the virus. So far as the preservation or destruction of the virus in lesions and cultures concerned, our experiences are summarized as follows:

A. Virus in pleural exudates: Inoculation into cattle with pleural exudates in test tubes kept in the ice-chest at 3° – 15°C . showed their original virulence over six months, cultural tests showing the characteristic growth of the virus.

B. When serum broth, serum Martin's broth agar or serum agar cultures closed tightly and kept in an incubator (37° – 38°C .), in an ice chest (3° – 15°C .) or at a room temperature (5° – 32°C .), they retained their ability to develop as shown in the table.

Days kept Temperature($^\circ \text{C}$)	30	50	70	110	140
37° – 38°	+	$\pm$	-	-	-

5°-32°	+	+	+	±	-
3°-15°	+	+	+	±	±

As seen above, cultures kept in the incubator lost the ability to grow within about 50 days, while in those kept in an ice-chest at a temperature not exceeding 15° C. and at a room temperature the attenuation of the virus occurred earlier than 110 days. However, its vitality was not regular as shown here even under the same conditions. In a word, it was, as a rule, found necessary to transfer every one month for keeping the culture in the laboratory.

Resistance to heat.

Moist heat: The serum broth culture was closed in glass tubes and heated in a water bath at a definite temperature, and then cooled as soon as possible and cultivated.

Time Temperature(C)	2	5	10	20	30	60	120	180	240
45°	+	+	+	+	+	+	+	+	-
50°	+	+	+	+	±	-	-	-	-
55°	+	-	-	-	-	-	-	-	-
60°	-	-	-	-	-	-	-	-	-

From the above table, it will be seen that the virus (culture) was destroyed in 2 minutes at a temperature of 60° C., in 5 minutes at 55° C., in 1 hour at 50° C., and in 4 hours at 45°.

Dry heat: Pieces of a sterilized porcelain filter (Chamberland) which were immersed into a thick emulsion of the culture were dried in a desiccator overnight. Pieces thus treated were exposed to a definite temperature for a certain length of time, and then were placed in culture media.

As shown below, the virus showed more resistance to dry than to moist heat.

Time Temperature(C.)	5'	10'	20'	30'	1 ^h	2 ^h	3 ^h	4 ^h
45°	+	+	+	+	+	+	+	-
50°	+	+	+	+	+	+	-	-
55°	+	+	+	+	+	-	-	-
60°	+	+	+	+	+	-	-	-
65°	+	+	+	+	-	-	-	-
70°	+	-	-	-	-	-	-	-

Resistance to chemical disinfectants.

Eight chemical disinfectants were used for the examination of destructive action on the virus. These were lysol, creolin, cresin, chlorate of lime, sublimate, carbolic acid, formalin and alcohol. The double concentrated solutions were previously prepared, and the equal part of these solutions and of virus suspension was mixed and at a temperature of 17°—20° C.

The results obtained are following :

Sublimate solution (1 : 5000), 0.1% chlorate of lime, 1% cresin, lysol and creolin have a most powerful disinfecting action, the virus being destroyed by short exposing. Formalin and carbolic acid has a weaker action, 0.5% carbolic acid destroying the virus within three hours, and 4 % formalin (4 % solution of the formalin shown in pharmacopoeia Japonica) in 20 minutes.

SUMMARY AND CONCLUSIONS.

From the present knowledge it is clear that the presence of oxygen in culture tubes is absolute necessity in growing the virus as insisted by Dahmen. So, in shake or stab cultures (deep serum agar) the virus develops at a definite distance from the surface and downward, forming a zone of growth or a continuous streak. Moreover, as a further aid in cultivation, tight closing of tubes with rubber stoppers was found to be convenient and satisfactory.

[illegible]

Disinfectant	Sublimate												
Time Per Cent	0.5'	1'	2'	5'	10'	20'	30'	1h	2h	3h	4h	5h	6h
0.002	+	+	+	+	+	+	+	+	+	+	+	+	+
0.01	+	-	-	-	-	-	-	-	-	-	-	-	-
0.02	-	-	-	-	-	-	-	-	-	-	-	-	-

Disinfectant	Alcohol												
Time Per Cent	0.5'	1'	2'	5'	10'	20'	30'	1 ^h	2 ^h	3 ^h	4 ^h	5 ^h	6 ^h
16	+	+	+	+	+	+	+	+	+	+	+	+	+
32	+	+	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	-	-	-	-	-

The addition of blood serum to media adapted is an important factor as several investigators have recommended, and it is found that 10 to 15 per cent blood serum was sufficient for luxuriant growth which does not depend upon the kind of blood serum. However, the blood serum, fresh or treated below 70° C. is much better than that heated at a higher temperature. As regards the reaction the suitable hydrogen ion concentration ranges from 6.0 to 8.5, the growth being luxuriant at pH. 7.5. The optimum temperature is 36° to 38° C; the growth ceases below 27° C.

An easy growth is obtainable on 10 per cent serum agar (3% agar). When 0.5 per cent sugar (for example 0.5 per cent glucose, serum agar) was added to the serum agar, the growth was much better. When incubated for a long time the colonies increased in size and showed a thick part in the centre, frequently two or more, taking various irregular forms later on. In general, the development of the colony in the first generation is faint, being

easily overlooked, but by generations it becomes to grow with ease. Furthermore, for growing the virus passed through a filter a large quantity of it should be used; otherwise, it is hardly successful. Acid production in the course of development of the virus was recognized by several authors. Our experiments showed serum fuchsin agar with 0.5 per cent glucose, mannose or maltose to be suitable for the purpose, reddening of the medium being very pronounced within 5 or 7 days. When closed in glass tubes and kept at a temperature of 3°–15° C., pleural exudate retains its virulence over six months. At 37°–38° C. cultures are destroyed within 50 days, and at a room temperature (5°–32° C.) or a lower temperature (3°–15° C.) they are alive for 70 days. The virus showed less resistance to moist than to dry heat. Of disinfectants, 0.1% chlorate of lime, 1% cresin, lysol and creolin, and sublimate (1:5000) destroyed the virus in a short time, while formalin and carbolic acid showed less destructive action.

As a result of those studies, the following conclusions are drawn:

1. The best medium for growing the virus of contagious pleuro-pneumonia was found to be an agar medium with 10 per cent serum, having a pH of 7.5.

2. A certain quantity of oxygen necessary for the growth of the virus was aimed by closing culture tubes with rubber stoppers.

3. The colonies were not constant in shape as described in several literatures, various structures being observed in the course of time.

4. For keeping the culture it was found necessary to transfer every one month, vitality being irregular even under the same conditions.

5. The virus showed less resistance to chemical disinfectants. Sublimate 1:5000, 0.1% chlorate of lime, 1% lysol, creolin and cresin as well as 2% carbolic acid may be used for disinfection of infected stable, etc.

6. There was no difference in biological characters between five strains employed.

LITERATURES.

(1). Josep, K.: Zur Züchtung des Lungenseucheerregers. *B. T. W. Jahrg.* 38. 1922.

- (2). Giese: Zur Züchtung des Erregers der Lungenseuche der Rinder. Ditto.
- (3). Derselbe: Die Ermittlung der Lungenseuche des Rindes mit Hilfe der Komplementablenkungsmethode. *B. T. W.* Jahrg. 37, 1921.
- (4). Kurt Poppe: Untersuchungen über die experimentelle Diagnose der Lungenseuche des Rindes. *Arb. a. d. Kaiserlichen Gesundh.* 45. B. 1913.
- (5). Giese & Wedemann: Zur Feststellung der Lungenseuche beim lebenden Rinde. *Arb. a. d. Reichsgesundh.* 55 Bd, Heft I, 1924.
- (6). Heslop: Further researches into the serological diagnosis of contagious pleuro-pneumonia of cattle. *J. Comp. Path. and Therap.* Vol. 35 No. 1. 1922.
- (7). Dahmen: Beitrag zur Studium der Lungenseuche des Rindviehs. 2. Mitteilung. *Arch. d. Wiss. u. Prakt. Thlkd.* Bd. 49, 1913.
- (8). Dujarnin-Beaumetz: Die Peripneumonie der Rinder. *Handb. der Pathog. Micro.* von Kolle-Wassermann, 2. Aufl. Bd. 8. 1913.
- (9). Huttyra & Marek: Lungenseuche der Rinder. in *Spezielle Path. und Therap. der Haust.* 1922.
- (10). 小野定志: 日本獸醫學會雜誌第4卷第1號及第3號 大正14年
- (11). 同: 中央獸醫學會雜誌第38輯5卷 大正14年

牛肺疫病毒ニ對スル生物學的研究補遺

農學博士 中村 哲 哉 二村彦治郎 渡瀬利夫

(農林省獸疫調査所)

牛肺疫病毒ノ人工培養ニ關シテハ先人ノ發表スル研究報告尠カラズシテ其ノ發育ニ對スル幾多ノ要約明ニセラレタリト雖モ未ダ其ノ完全ヲ期シ難キモノ、如シ且ツ物理化學的感作ノ本病毒ニ對スル影響ニ付テハ闡明セラレサル點頗ル多ク從ツテ實際上本病毒ノ尅滅ニ資スベキ方寸ノ確定ヲ期シ難キノ憾アリ仍ツテ昨夏本病ノ初メテ内地ニ發生スルヤ自ラ之ガ生物學的性質ヲ一層明ニスルノ必要ニ遭遇シ先ヅ先人ノ之ニ關スル業績ヲ追試スルト共ニ本病毒ノ培養ニ關スル完全要約ノ探究ニ努メ併セテ其ノ溫熱及各種消毒藥ニ對スル抵抗力ノ強弱ヲ實驗シテ之ヲ實際の應用上ニ於ケル本病毒ノ合理的滅殺ヲ期セリ今本試驗ニ於テ得タル結果ヲ綜合スレバ大要次ノ如キ結論ヲ下シ得ベシ

1. 牛肺疫毒ノ發育ニ適スル培養基ハ 10% 血清寒天ニシテ且

1

2

3

4

5

6

8

9

10

10

11

12

ツ其ノ水素いおん濃度ハ7.5ヲ呈スルモノトス

2. 本病毒ノ試験管内發育ニハ一定量ノ酸素アルヲ要シ且ツ所要温度ノ保存ノ爲移植後ごむ栓ニテ封スルヲ可トス

3. 發育集落ノ形狀ハ常ニ一定セルモノニ非ズシテ培養期間ノ延長ニ從ヒ諸種ノ肉眼の構造ヲ呈ス

4. 培養病毒ノ生存期間ハ同一狀態ニアリテモ常ニ一定セザルヲ以テ毎月之ヲ移植スルヲ要ス

5. 本病毒ノ化學的消毒藥ニ對スル抵抗力ハ比較的弱ク 5,000倍昇汞水, 0.1%くろーる石灰, 1%ノりぞーる, くれをりん, くれしん 2%竝石炭酸ハ本病毒汚染牛舎等ノ消毒ニ有効ナリ

(自抄)

EXPLANATION OF PLATE.

All the figures were taken with the "PHOKU" camera using a "a," lens.

Fig. 1-3: The typical shapes of colonies (within 7 to 10 days). Fig. 4-12: Various structures of colony observed in the course of time (within 3 to 5 weeks).

Fig. 4. A colony with a less thick periphery.

Fig. 5. Colonies with a thick moist transparent periphery.

Fig. 6. An umbrella-shaped colony with radiating structure.

Fig. 7. Colonies with a thick, wrinkled periphery.

Fig. 8. The thick centre of colonies left by the disappearance of the periphery.

Fig. 9. and 10. An abnormal appearance of colonies with numerous granules over them.

Fig. 11. An enlarged colony with a pitted surface.

Fig. 12. An enlarged colony with a smooth surface.