ON THE ANTIGEN FOR COMPLEMENT FIXATION TEST IN CONTAGIOUS PLEURO-PNEUMONIA IN CATTLE.

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INTRODUCTION.

In spite of the unparalleled variety of works concerning the contagious pleuro-pneumonia in cattle, the detection of this disease by a reliable and practically applicable method remains as the most important problem. On the sudden outbreak of this disease in our country, a comparative study of several methods regarding serological diagnosis was made and it was found that the complement fixation test was most reliable as a supplement measure for the earlier detection of this disease. Since the first trial of this test as a diagnostic for this disease was made by Schochowsky in 1913, several investigators carried out experiments using various antigens in order to examine the practical value of this test for the same purpose. Shortly, it may be said that the determination of the practical value of this test depends largely upon the specificity and potency of antigen to be used. In view of the publication made by several workers up to the present time, we can say that the distilled water extract of lung lesions taken from acute cases as Titze and Giese employed, and cultures in glucose-serum-broth made by Giese and Wedemann, may be used in practice as the most valuable antigen. Having prepared 13 kinds of antigen, we carried out our experiments to study the antigenic character of the virus of contagious pleuro-pneumonia strains at hand and have obtained the following results.

ANTIGENIC PROPERTY OF VARIOUS KINDS OF PREPARATION.

A. Antigenic value of the culture of the virus.

1. Serum agar culture.

A 10-day-culture on serum-agar was emulsified with salt solution, sometimes heated but sometimes not heated, and then the antigenic property was tested. None of them was found to be suitable for practical use because of marked anticomplementary effect and lack of specificity.

Now, further examination was made to study the antigenic value of the turbid condensation water and the pure emulsion of colonies grown on surface media.

a. Emulsified colonies.

All the colonies grown on various serum-agars (3%), added with varying quantities of horse serum (1, 2, 5, 10, and 20%) for 10 days, were respectively emulsified in about 3c.c. of salt solution after complete removal of the condensed water, and were divided into three parts; two parts being treated by heating at 60°C. for one hour and at 100°C. for 10 minutes respectively, the remaining part being not treated.

Each of them was added with 0.5% carbolic acid, and was preserved. The results obtained by using these antigens are tabulated as follows:—

Antigen	Percentage of serum added	Object of examination		Dose of antigen used							
				0.5	0.3	0.2	0.1	0.08	0.05	0.02	
Non-heated emulsion	1 %	Anticmpl.-property		±	+	++	+++	+++	+++	+++	
		Antigenic property.	Infected serum				-	±	+	+	
			Normal serum				++	+++	+++	+++	
	2 %	Anticmpl.-property		±	+	++	+++	+++	+++	+++	
		Antigenic property.	Infected serum				-	-	±	+	
			Normal serum				++	+++	+++	+++	
	5 %	Anticmpl.-property		±	+	++	+++	+++	+++	+++	
		Antigenic property.	Infected serum				-	-	±	+	
			Normal serum				++	+++	+++	+++	
	10%	Anticmpl.-property		-	+	+	++	+++	+++	+++	
		Antigenic property.	Infected serum				-	-	+	+	
			Normal serum				+	++	+++	+++	

	20%	Anticompl.-property		-	±	+	+	+	+	+	+
		Antigenic property	Infected serum				-	-	±	+	+
			Normal serum				+	+	+	+	+
Emulsion heated for one hour at 60°C.	1 %	Anticompl.-property		+	+	+	+				
		Antigenic property	Infected serum		-	-	-	+	+	+	+
			Normal serum		+	+	+				
	2 %	Anticompl.-property		+	+	+	+				
		Antigenic property	Infected serum		-	-	-	+	+	+	+
			Normal serum		+	+	+				
	5 %	Anticompl.-property		+	+	+	+				
		Antigenic property	Infected serum		-	-	-	±	+	+	+
			Normal serum		+	+	+				
	10%	Anticompl.-property		-	+	+	+				
		Antigenic property	Infected serum		-	-	-	±	+	+	+
			Normal serum		+	+	+				
	20%	Anticompl.-property		-	+	+	+				
		Antigenic property	Infected serum		-	-	-	±	+	+	+
			Normal serum		+	+	+				
Emulsion heated for 10 minutes at 100°C.	1 %	Anticompl.-property		+	+	+	+				
		Antigenic property	Infected serum	-	-	-	+	+	+	+	+
			Normal serum	+	+	+	+				
	2 %	Anticompl.-property		+	+	+	+				
		Antigenic property	Infected serum	-	-	-	±	+	+	+	+
			Normal serum	+	+	+	+				
	5 %	Anticompl.-property		+	+	+	+				
		Antigenic property	Infected serum	-	-	-	±	+	+	+	+
			Normal serum	+	+	+	+				
		Anticompl.-property		+	+	+	+				

10%	Antigenic property	Infected serum	-	-	-	±	+	+	+
		Normal serum	+	###	###	###			
20%	Anticmpl.-property		+	###	###	###			
	Antigenic property	Infected serum	-	-	-	±	+	+	+
		Normal serum	+	###	###	###			

Remarks : ### denotes Complete haemolysis, ## Almost complete haemolysis, + Half haemolysis, + Slightest inhibition, ± Almost complete inhibition, - Complete inhibition.

As shown in the above table, the non-heated emulsion was markedly anticomplementary and less specific, while the heated emulsion was proved to be highly specific.

It is a matter of owre that the antigenic value may be interfered with by heating; so the boiled emulsion may become less potent as regards antigenic power. In order to examine the effect of horse serum protein upon the emulsion, each of the emulsions prepared from colonies on the surface of media was filtered through Mandel's filter, and the filtrates were diluted with salt solution in proportions of 1:2, 1:4..... and so on and then added with the precipitin serum for horse flesh. Positive reactions were obtained where the emulsion with 1 per cent. serum agar in a dilution of 1:8, the emulsion with 2 or 5 per cent. serum agar in a dilution of 1:16 and the emulsion with 10 or 20 per cent. serum agar in a dilution of 1:32 were employed respectively. Therefore, the effect of the horse serum protein in the non-heated emulsion seemed to be dependent upon its quanti, and the elimination of such protein can be aimed by heating process.

Now, in order that if an antigen be of value, the antigenic dose must be considerably smaller than the anticomplementary quantity determined. The determination of the suitable doses of antigen which was prepared from the colonies on 5 per cent serum agar by heating at 60°C. for one hour, was carried out as shown in the next table.

Dose of antigen used. (c.c.)	Serum	Dose of serum tested. (c.c)							
		0.1	0.05	0.02	0.01	0.005	0.002	0.001	
	Infected serum	-	-	-	-	-	-	-	-

0.3	Normal serum	+++	+++	+++	+++			
	Infected serum	—	—	—	—	—	—	—
0.2	Normal serum	+++	+++	+++	+++			
	Infected serum	±	—	—	—	+	+++	+++
0.1	Normal serum	+++	+++	+++	+++			
	Infected serum	++	+	+	+	++	+++	++
0.08	Normal serum	+++	+++	+++	+++			
	Infected serum	+++	+++	+++	+++			

As will be seen, the antigenic value of the emulsion made from colonies on solid media is prominent, the emulsion being thereby heated at a certain temperature (60°C.) for 4–5 hours. As regards the quantity of serum to be added to media, 3–10% seemed to be most favourable for the luxuriant growth of the virus, the reaction being kept at Ph 7.5.

b. Condensation water of serum-agarcultures.

The condensation water was collected from 10-day-old serum-agar cultures added with varying quantities of horse serum such as 1, 2, 5, 10 and 20 per cent., and they were divided into three parts and tested as in the previous case.

The results showed that the antigenic power of the condensation water was unrecognizable. Observing the growth of the virus in condensation water, it may be supposed that any serviceable antigen should exist there, as the development is remarkable even from the beginning of cultivation and becoming luxuriant in the course of time. If the result of this experiment be sure, the practical value of the antigen can not be estimated only from the development of the virus.

2. Glucose-serum-bouillon culture (*Giese's antigen*).

A 50-day-old culture in the serum-glucose-bouillon (0.5 % glucose) added with the varying quantities of horse serum such as 2, 5, 10, 20, 30 and 50 per cent. was heated for 4 hours at 60°C. and then added with 0.5 per cent. carbolic acid. The colour of the preparation was more or less different according to

the quantity of serum added; the one with 2 and 5 per cent. serum being translucent and yellowish brown, the other with 10, 20 and 30 per cent. serum more turbid, and that with 50 per cent. serum opaque with remarkable turbidity. The results of the determination regarding their anticomplementary and antigenic properties are tabulated as follows:—

Percentage of serum added	Object of examination.		Dose of antigen used.				
			1.0	0.5	0.3	0.2	0.1
2 %	Anticompl.-property		###	##	##	##	##
	Antigenic property	Infected serum	±	+	##	##	##
		Normal serum	##	##	##	##	##
5 %	Anticompl.-property		+	##	##	##	##
	Antigenic property	Infected serum		—	—	—	+
		Normal serum		+	##	##	##
10%	Anticompl.-property		+	##	##	##	##
	Antigenic property	Infected serum		—	—	—	±
		Normal serum		+	##	##	##
20%	Anticompl.-property		—	##	##	##	##
	Antigenic property	Infected serum	—	—	—	—	—
		Normal serum		+	##	##	##
30%	Anticompl.-property		+	##	##	##	##
	Antigenic property	Infected serum		—	—	+	+
		Normal serum		##	##	##	##
50%	Anticompl.-property		##	##	##	##	##
	Antigenic property	Infected serum	+	+	+	##	##
		Normal serum	##	##	##	##	##

As seen from the above table, the antigenic property was most remarkably shown when cultures in 5, 10 and 20 per cent. serum-glucose-broth were used, but in others it was not so pronounced. In the case of 50 per cent. serum-glucose-broth

culture the antigenic substance has not been demonstrated. However, the addition of 2 per cent. serum was proved to be enough to produce some useful antigen. At any rate, the antigenic value of serum-glucose-broth culture appeared to be interfered with by the development of the virus in the media. Furthermore, it has been found that the saccharose may be used for the same purpose.

3. *Serum-bouillon cultures.*

4. *Serum-Martin's pepton-bouillon cultures.*

In both cultures the antigenic property was proved to be less marked as compared with that in the above described cultures.

5. *Serum-pepton-water-agar cultures.*

In this media the development of the virus was not good, and the anticomplementary property of the culture was remarkable, though the antigenic property could be more or less recognizable. This culture was therefore not worthy to be used as antigen.

6. *Emulsified colony with the serum-Martin's pepton culture.*

The above mentioned results obtained from the determination of antigenic value of various cultures suggested that the use of serum-Martin's pepton culture may render the antigenic value of any preparation much higher. Starting from this consideration, a comparative study was carried out, but the result was unsatisfactory, because the antigenic power of the new preparation was found to be insufficient.

B. Extract of affected lesions from the diseased animal.

1. *Extract of lung lesions. (Titze & Giese's antigen)*

A portion of hepatized lung taken from acute cases was finely chopped and extracted at 100°C. in 10 volumes of distilled water for half an hour. This was then centrifuged and the supernatant liquid was placed in a sterilized bottle into which 0.5 per cent. carbolic acid and 0.85 per cent. sodium chloride added. This extract was heavily turbid with a colour of grayish-

white. The results obtained are shown in the following table:—

Determination of anticomplementary and antigenic doses.								
Object of examination		Dose of antigen						
		0.5	0.3	0.2	0.1	0.08	0.05	0.02 0.01
Anticomplementary property		—	+	##	##			
Antigenic property	Infected serum		—	—	—	±	+	##
	Normal serum		+	##	##			
Determination of the suitable dose of the antigen.								
Doses of antigen	Serum tested	Doses of serum						
		0.1	0.05	0.02	0.01	0.005	0.002	0.001
0.2	Infected serum	—	—	—	—	—	—	+
	Normal serum	+	##	##	##			
0.1	Infected serum	—	—	—	—	—	+	##
	Normal serum	##	##	##	##			
0.08	Infected serum	+	+	—	—	+	+	##
	Normal serum	##	##	##	##			
0.05	Infected serum	##	+	+	+	##	##	##
	Normal serum	##	##	##	##			

From the results described in the above table, it will be seen that the extract contained substances which in combination with the serum taken from the animal suffering from contagious pleuropneumonia possessed a power of fixing complement and 0.1c.c. of the extract was suitable for use. Now, it will be necessary to determine the behavior of normal serum which might show a false reaction with the mentioned extract. For this purpose, a large number of serum specimens taken from the normal cattle (120 cattle) were tested. The results obtained showed that the anticomplementary property of the normal serum has exerted almost no influence upon the test, but the inhibitory action of the extract was somewhat interfered with by the presence of 0.02–0.01c.c. of

the normal serum in 20 to 50 per cent. of the cases. For this reason, the antigenic value of the extract seemed to be less potent as compared with that of the serum-glucose-bouillon culture or the emulsified colonies.

2. *Extract of bronchial lymphatic glands.*
3. *Alcoholic extract of lung lesions or bronchial lymphatic glands.*
4. *Pleural exudate taken from affected cattle.*

These extracts and the material were found to be of use, but not so prominent.

As already described above, the antigenic value of both glucose-serum-bouillon culture and emulsified colonies has been determined to be prominent. In order to study these antigens more closely further examination was carried out, especially on the relation between the incubation period and the antigenic value of culture.

It is important for practical work to ascertain, if the antigenic value of culture would be influenced by its incubation period. For this purpose, the serum-agar and serum-glucose-bouillon cultures of the virus were examined at varying periods of incubation from one to eight weeks in the same way as above mentioned.

The emulsion of the colonies grown luxuriantly on the surface of serum agar was found to have high antigenic value, but any recognizable difference regarding the value was not observed among several cultures at different lengths of cultivation. It has been found, moreover, that one-week-old culture could not be used, because of want of material, and the cultures over two weeks were also not suitable for use, because various kinds of bacteria happened to grow in spite of carrying out the technic under minute precaution. For this reason, we may say that it is practically recommendable to prefer 10-14-day-old cultures to others for the preparation of a potent antigen.

As for the serum-glucose-bouillon culture 4 weeks old one was found, as a rule, to be suitable for use, if the growth be luxuriant, though the fact that a decrease of antigenic power always takes place in over 8-weeks-old glucose-serum-bouillon culture was

pointed out by Giese and Wedemann in 1924. Our 14-weeks-old fluid culture gave invariable results on the titration of its antigenic power. Nevertheless, it seemed to be unable to obtain an antigen with almost equal potency. In order to know the effect of preservation period upon the antigenic value, the titrated antigens were tightly sealed in the bottle preventing from day light, and preserved at room temperature. They were then examined at intervals of 14-28 days during 16 months, but no decrease in antigenic power was noticed. It must be pointed out here that from our experiments a highly stable antigen could be obtainable, if the emulsion be kept for several weeks after preparation.

ANTIGENIC VALUE OF CULTURE-FILTRATE.

The cultures were centrifugalized or filtered through Chamberland filter F or Mandel's filter, and some of old cultures were settled; the clear supernatant portion, filtrates and sediment were all used for the determination of antigenic value. For the titration, we employed sera which showed such higher titres, as 0.005 and 0.002.

Emulsified colonies									
Antigen tested	Kind	Object of examination		Dose of antigen					
				0.5	0.3	0.2	0.1	0.08	0.05
A antigen (three days after the production)	Original emulsion	Anticompl.-property		—	+++	+++	+++		
		Antigenic property	A. serum		—	—	—	—	—
			B. serum		—	—	—	—	—
			C. serum		—	—	—	—	—
	Supernatant portion (centri- fugalized)	Anticompl.-property		++	+++	+++	+++		
		Antigenic property	A. serum	—	—	—	—	±	+
			C. serum	—	—	—	—	—	—
	Filtrate through Mandel's filter	Anticompl.-property		+++	+++	+++	+++		
		Antigenic property	A. serum	++	+++	+++	+++	+++	+++
			B. serum	+++	+++	+++	+++	+++	+++
			C. serum	—	—	—	±	+	+

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B antigen (13 months after the production)	Filtrate through Chamberland's filter	Anticompl.-property		###	###	###	###		
		Antigenic property	A. serum	##	+	+	##	+	###
			B. serum	###	###	###	###	###	##
			C. serum	±	±	±	±	±	±
	Original emulsion	Anticompl.-property		+	###	###	###		
		Antigenic property	A. serum	-	-	-	-	-	-
			B. serum	-	-	-	-	+	+
			C. serum	-	-	-	-	-	-
	Supernatant portion (centri- fugalized)	Anticompl.-property		##	###	###	###		
		Antigenic property	A. serum	-	-	-	-	±	+
			B. serum	-	-	±	##	##	##
			C. serum	-	-	-	-	-	-
Filtrate through Chamberland's filter	Anticompl.-property		###	###	###	###			
	Antigenic property	A. serum	##	###	##	##	###	##	
		C. serum	##	##	+	+	##	##	
Precipitate	Anticompl.-property		-	+	+	+	##	##	
	Antigenic property	A. serum		-	-	-	-	-	
Glucose-serum-bouillon culture									
				Dose of antigen					
				1.0	0.5	0.3	0.2	0.1	0.08
C antigen (6 days after the production)	Original culture	Anticompl.-property			+	###	###	###	##
		Antigenic property	A. serum		-	-	-	+	+
			B. serum		-	-	-	+	##
			C. serum		-	-	-	-	-
	Supernatant portion (centrifuga- lized)	Anticompl.-property		-	##	###	###	###	##
		Antigenic property	A. serum		-	-	+	##	
			B. serum		-	-	+	##	
			C. serum		-	-	-	+	

Filtrate through Mandel's filter	Anticompl.-property		+++	+++	+++	+++	+++	+++
	Antigenic property	A. serum	+++	+++	+++	+++	+++	+++
		B. serum	+++	+++	+++	+++	+++	+++
		C. serum	+++	++	+++	+++	+++	+++
	Anticompl.-property		-	++	+++	+++	+++	+++
Precipitate	Antigenic property	A. serum		-	-	-	±	+
		B. serum		-	-	-	-	-

As seen from the above table, the supernatant portion was less antigenic and that of the filtrates was least. Therefore, the use of such antigens for the complement fixation test would not be effective. On the contrary, the sediment which settled to the bottom of the bottle was proved to be more anticomplementary as well as antigenic than homogeneous suspension. From these observations, it may be said that the virus itself might have a specific antigenic power. Furthermore, it will be of great importance that the suitable dose of antigen to be used for the diagnosis of this disease must be titrated by employing a large number of sera taken from the diseased and normal animals as well. In other words, the serviceable antigen must in any case be as potent as to have the power of absorbing complement in a specific way in the presence of the corresponding amboceptor.

ANTIGENIC VALUE OF VARIOUS STRAINS OF THE VIRUS.

As described in the previous work, the various strains of the virus of contagious pleuro-pneumonia from a number of cases showed no difference regarding their biological characters, but the antigenic value of each strain is not yet studied. If there be any difference in antigenic character, selection of strain for the preparation of antigen would be of great importance.

Of course, in the course of the previous work "On the practical value of several serological reactions for the diagnosis of contagious pleuro-pneumonia in cattle", we have found the fact that some strain of the virus did not show any antigenic property. Based upon this fact, an attempt was carried out to ascertain if

there be any difference in the antigenic power according to the different source of virus strain. Nine strains of the virus isolated from infected animals and one strain obtained from the "Lungens-euche-Lymphe Hoechst a. M." were used for the experiments. The results showed that the antigenic value of eight strains was almost equivalent, but there was no evidence of antigenic character in two other strains (F and D strain). However, it was noticed that after generations of culture (about 6 to 11 months) the antigenic power of F and D strains became so strong that they could be used as antigen.

On account of such difference regarding antigenic property some effort must be made to select the strain for the production of an effective antigen.

ANTIGENIC VALUE OF EMULSIFIED COLONIES AND GLUCOSE-SERUM-BOUILLON CULTURE.

As already understood, the antigenic power of the emulsified colonies and that of glucose-serum-bouillon culture were both proved to be very potent; however, in respect to the specificity and potency, the former was shown to be more excellent.

We have had the opportunity to test comparatively the applicable value of both antigens in a large number of cases (3000-6000 blood specimens sent from Kyoto Prefecture every month). Two cases gave positive reaction with the former antigen while one of them showed slight inhibitory action and the other negative reaction with the latter one. On the postmortem examination, both cases revealed the same findings, the liquefied necrotic foci, an apple to a head in size, surrounded by a thick layer of connective tissue, were observed in the anterior or posterior lobe of the right lung, and the pleural surface of the affected side was also found to be thickened by the formation of connective tissue. In 1926, Karmann and Witte proposed that if both active and inactive sera would be tested using Giese's antigen, the reaction will be much reliable for the detection of the disease in living animals.

Now, from a series of experiments following this proposal, the next results were obtained.

No. of serum		Antigen	Dose of serum							
			0.1	0.05	0.02	0.01	0.005	0.002	0.001	
573 serum	Inactive	Anticomplementary	—	—	—	—	卄	卄	卄	
		C antigen	—	—	卄	卄	卄	卄	卄	
		Anticomplementary effect of serum	卄	卄	卄	卄				
	Active	A antigen	—	—	—	—	±	卄	卄	
		C antigen	—	—	—	+	卄	卄	卄	
		Anticomplementary effect of serum	+	卄	卄	卄				
609 serum	Inactive	A antigen	—	—	±	卄	卄	卄	卄	
		C antigen	卄	卄	卄	卄	卄	卄	卄	
		Anticomplementary effect of serum	卄	卄	卄	卄				
	Active	A antigen	—	—	—	±	卄	卄	卄	
		C antigen	—	—	—	±	卄	卄	卄	
		Anticomplementary effect of serum	卄	卄	卄	卄				

Remarks: "A" antigen denotes emulsified colonies. "C" antigen denotes glucose-serum-bouillon culture (Giese's antigen).

So far as the results shown in the above table concerned, both active sera showed to be more powerful in fixing complement in the presence of glucose-serum-bouillon culture antigen. However, it was also recognized by these workers, as well as by others that active normal sera showed occasionally a marked haemolytic-inhibitory effect or was haemolytic in itself. According to our experiments regarding this point, there were many cases in which active normal sera manifested a higher anticomplementary power or were proved to be haemolytic in itself. On account of these facts, if such sera be tested, a non-specific complement fixation or a false negative reaction will often take place with the given antigen. In these cases the anticomplementary property or haemolytic power can usually be destroyed by heating them at 56°C. for half an hour, though occasionally even inactivated sera may remain slightly anticomplementary or haemolytic. Of course, these controls are indispensable in every case in which inactivated

sera are examined. For this reason, if the complement fixation test will be conducted as a supplementary measure for the diagnosis of contagious pleuro-pneumonia, we prefer to use our emulsified-colonies-antigen, the serum tested being inactivated.

CONCLUSION.

1. The emulsion made from colonies on solid media was most specific and potent in its antigenic power, though the glucose-serum-bouillon culture could also be used as a valuable antigen.

2. So far as the preservation of both antigens at room temperature was limited to 16 months, they remained usually to be potent.

3. Both antigens, if centrifugalized or filtered, the supernatant portion or filtrate obtained was proved to be less or not to be potent. Therefore, it may be supposed that the virus itself might have a specific antigenic power.

4. It is highly probable that there is some strain not to be serviceable for the production of specific antigen, but even such strain can be rendered as potent as other useful strains, if submitted to several generations of culture.

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二村 彦 治 郎 渡 櫨 利 夫

(農 林 省 獸 疫 調 査 所)

(昭 和 2 年 10 月 29 日 受 附)

牛肺疫生前診断ニ於ケル補體結合反應ノ應用的價值ハ試験ニ使用スルあんちげんノ撰擇ニ著シキ關係アルノ事實ニ鑑ミ專ラ特異性ニ富ムベキあんちげんノ製造ヲ企テ既ニ有効ナリトシテ Titze & Giese 及 Giese & Wedemann ノ推獎スル 2 種あんちげんノ外本病毒ノ培養又ハ病的材料ヨリ造レル總計 13 種ノあんちげんニツキ各特異性ヲ比較檢定セシ結果ハ本病毒ノ寒天斜面發育集落食鹽水乳劑ヲ 60°C. ニ加温處置セルモノニ於テ特異性高キヲ認メタリ又大正 14 年以來本病ノ發生地ニ於テ採收セル材料竝ヘキスニ製牛肺疫豫防液ヨリ分離セル病毒 10 株ノ培養ヨリ造レルあんちげんノ効力ヲ檢査セシニ内 2 株ニ於テハ分離後一定期間其ノ培養ニ特異あんちげん性ナキヲ認メタリ尙本試験ニヨリ病毒ノ人工培養ニ缺ク可カラザル馬血清ノ培地添加量及病毒培養期間等ハ直接ニあんちげん製造量ノ多寡及其ノ特異性ノ強弱ニ影響ヲ來スヲ認メ且特異あんちげんハ主トシテ病毒自體ニ存スヘキヲ明カニセリ又該集落乳劑あんちげんハ製造後 16 箇月室温ニ保存スルモ毫モ其ノ特異性ヲ失フコトナキヲ知レリ

(自抄)