ON THE HEMOTOXIN OF THE SARCOSPORIDIA

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In literature, the studies on the activators in connection with this toxin, in especial reference to hemolysis, such as lecithin or serum, as they have been used in hemolysis by snake venoms or other sorts of phyto-toxins, have been scarce. Only the papers accessible to me were those of Teichmann⁽¹⁾ and Cominotti⁽³⁾, who tested the hemolytic action of sarcosporidiotoxin against the erythrocytes of the rabbit, sheep and horse. They all report a negative result. Teichmann and Braun⁽²⁾ later report that in this toxin they proved hemagglutinative principle.

As was reported in my preliminary communications⁽⁴⁾, I have proved on the hemagglutinative and hemolytic principles in the sarcosporidiotoxin, which consisted of the dessicated specimens of the *Sarcocystis blanchardi* found parasitic in the Formosan buffaloes. Concerning the facts that a very scrupulous care is necessary in preparing the infusum; that the results obtained by the use of the erythrocytes of various species of animals are divergent; that it has its attitude toward sera and their components, especially lecithin, cholesterol and serum proteins, and that it has specific physico-chemical and serological natures, this toxin would seem widely different from other zootoxins. The results are dealt with in this paper.

Physico-chemical Natures

The hemolytic principle, which is found in the sarcosporidiotoxin is alcohol and ether soluble, but hardly soluble in either distilled water or physiological saline. It would seem very probable that

it has something of a colloidal construction. It can hardly be demonstrated, if the technique of the preparation of the infusum, especially the maceration should be imperfect. The hemolytic principle of the toxin, again, is thermostabile but if the freshly prepared material should be heated at 65°C. or above the principle in question will be brought down with the precipitates. This is another point of notice in the demonstration of the hemolytic activity of this toxin. In the following Table I, the prominent characteristics of the hemagglutinative and hemolytic principles are shown :

Table I

Natures	Hemolytic principle	Hemagglutinative principle
Solubility: in distilled water	Hardly soluble	Soluble
" the saline	Hardly soluble	Soluble
" glycerine water	Hardly soluble (action is interrupted by glycerine)	Soluble (action is not interrupted by glycerine)
" alcohol-water	Soluble	Insoluble
" ether-water	Soluble	Insoluble
Resistances: against heat	Stabile up to 120°C, 2 hours	Destroyed by 85°C, 1/2 hour
" acid	Comparatively strong against acid, but weak against alkalies	Strong against acid but very weak against alkalies
Dialyzation:	Not dialyzable	Not dialyzable
Adsorption:	Adsorbed by animal charcoal and caorin	Adsorbed by animal charcoal and caorin
Protein reactions:	Negative	Positive

Action of the Hemotoxin Alone against the Washed Erythrocytes

Hemolytic and Hemagglutinative Actions: The sarcosporidiotoxin has divergent strengths of both hemolytic and hemagglutinative actions against the washed erythrocytes of animals, which are shown in the following Table II:

Table II

Sources of erythrocytes (in a 5% suspension)	Toxin alone		With 20% serum		With lecithin	
	Heml.*	Hemag.**	Heml.	Hemag.	Heml.	Hemag.
Guinea-pig	3 200- 6 400	25 600	± (200)	51 200 (raised)	51 200 (raised)	Uninfluenced if not raised

Horse	1 600- 3 200	51 200	—	102 400 (raised)	25 600 (raised)	Somewhat lowered
Rabbit	800- 1 600	102 400	—	Uninfluenced	640 (raised)	do.
Goat	800- 1 600	12 800	—	do.	12 800 (raised)	Uninfluenced
Pig	800 1 600	51 200	—	do.	6 400 (raised)	do.
Dog	400- 800	51 200	—	do.	6 400 (raised)	Somewhat lowered.
Buffalo	400- 800	204 800	—	do.	3 200 (raised)	Uninfluenced if not lowered
Human	400- 800	102 400	—	do.	6 400 (raised)	12 800 (mar- kedly lowered)
Chicken	400	51 200	—	320 (lowered)	1 600 (raised)	12 800 (lowered)
Pigeon	400 ?	51 200	—	12 800 (lowered)	800 ?	3 200 (lowered)
Duck	400 ?	102 400	—	51 200 (lowered)	800 ?	12 800 (lowered)
Sheep	400 ?	12 800	—	102 400 (raised)	?	102 400 (raised)
Cattle	400 ?	25 600	—	51 200 (raised)	?	Uninfluenced
Formosan zebu	400 ?	12 800	—	uninfluenced	?	do.

*Heml. stand for hemolysis and **Hemag. for hemagglutination.

From the above table, it will be seen that the hemolytic action was strongest against the erythrocytes of the guinea-pig among the erythrocytes of the 14 species of animals (1/6 400), next against those of the horse, rabbit, dog, pig, human and goat (1/3 200–1/1 600) while weakest against those of the buffalo, chicken, pigeon, duck, sheep, cattle and Formosan zebu (1/400). The hemagglutinative action was strongest against the erythrocytes of the buffalo (1/200 000), those of the human, duck and rabbit (1/100 000) stood next in order of strength while those of the dog, pigeon, horse, guinea-pig, sheep, cattle and Formosan zebu (1/50 000 –1/10 000) were the weakest.

The Optimal Concentration of the Erythrocytes for Two Reactions: The optimal concentration of the erythrocytal suspension for both hemolytic and hemagglutinative reactions was 2.5–5.0%. The weaker the concentration was, the wider was the range of the hyperinhibitory phenomena. Even when all the erythrocytes should

be laked, the freed hemoglobin would be sedimented and the proper tonus of the color were made obscure and accordingly the judgement of the results was difficult to be established.

Velocity of the Development of Both Reactions: The velocity of the development of the reactions was not simultaneous with both tests, because the hemagglutinative reaction developed by far the speedier than the hemolysis, or the former reaction was seen to develop in several minutes, and the complete phenomena took place in two hours, while hemolysis began to develop gradually in one half to one hour and the development of the complete phenomena took place in about 20 hours.

Fixation of Erythrocytes and the Stromata: The hemotoxin fixes both the erythrocytes and the stromata in vitro. The fixation developed quite indifferently with the species of the animals, i. e. the erythrocytes which were keen to the action of the hemotoxin absorbed just in a magnitude similar to the duller erythrocytes.

Influences of the Sera upon the Hemotoxin

Influence of Sera upon Hemolytic Toxin: The action of the hemolytic toxin is not accelerated by sera, whether they were homologous or inactivated heterologous or in whatever percentages they were used. If, however, the sera should be used in a very high percentage, (viz. 0.25 c.c. of a 20% solution), the hemolytic action was interrupted. The threshold dosis to inhibit the hemolysis against one unit of the toxin, i. e. 0.5 c.c. of a 1/400 dilution of the toxin, was 0.5 c.c. of a 1/20 dilution of the sera.

Action of the Heated Sera upon Hemolysis: The sera which had been heated at 65°–100°C. for one half hour, even had the inhibitory action against hemolysis. It has also been observed that the sera heated at 65°–80°C. had weaker inhibitory action than the fresh one, but those heated at 100°C. had almost as strong inhibitory action as the latter.

Action of Sera upon Hemagglutination: Allowing a very slight dissemination arising from the use of different methods of mixing or the different sorts of the sera, it has been generally observed that the hemagglutination was weakened by keeping the

mixture of the erythrocytal suspension and the sera at 37°C. for one half hour, but if the erythrocytes alone should be treated with the sera before the addition of the toxin, the hemagglutinative activity has been seen somewhat increased.

If the sera should be treated with 10-20 times their volume of pure alcohol, the extracts accelerated the hemolytic action, while the insoluble portion acted inhibitorily against both hemolysis and hemagglutination. By investigating the acceleratory and inhibitory substance by chemical view point, the following results could be learnt :

Substance	Reaction
Alcohol insoluble fraction of serum (protein).....	Inhibition of hemolysis very strong.
Globulin fraction.....	ditto weak ; hemagglutination strong.
Albumin fraction	ditto strong : ditto weak.
Alcohol soluble fraction of serum (lipoids).....	Hemolysis 4-8 times as strong as when toxin alone was used.
Aceton insoluble fraction (lecithin).....	Hemolysis 8 times as strong as when toxin alone was used.
Alcohol soluble fraction of lecithin	Hemolysis 16 times as strong as when toxin alone was used
Alcohol insoluble fraction (cephalin or quorin)	Hemolysis indifferent.
Aceton soluble fraction.....	Hemolysis 2 times as strong as when toxin alone was used.
Digitonin insoluble fraction (cholesterol)	Inhibition of hemolysis: trace.
Digitonin soluble fraction (neutral fats).....	Hemolysis 2 times as strong as when toxin alone was used.

From the above experimental data it was seen that :

(1) Amongst the alcohol soluble fraction in the serum, lecithin has the strongest power to accelerate the hemolysis by the sarcosporidiotoxin, while the neutral lipoids have a very strong power of autohemolysis, but very weak in accelerating the hemolytic power of the sarcosporidiotoxin. Cephalin and quorin are practically indifferent. It may be concluded, therefore, that hemolysis accelerating substance in the serum is lecithin fraction. Of all the methods of mixing lecithin, the mixing of the toxin, erythrocytes and lecithin, all at once gives the most vigorous action of hemolysis. This

accelerating action of lecithin is strongest in the fresh preparation but the longer the preparation is preserved, the weaker its action was, while the stronger the autohemolytic power was.

(2) The cholesterol fraction has been found practically indifferent for inhibitory action against hemolysis. I have obtained a negative results in my test in which I employed the saline suspension of gelatin, as the protective colloid, and cholesterol, Merck, as the inhibitory agent against hemolysis.

(3) The albumin fraction, which is one of the alcohol insoluble serum proteins, is the chief substance to inhibit hemolysis. The threshold amount of complete inhibition against one unit of the hemolytic toxin was 0.25 c.c. of a 1% solution of the dried alcohol insoluble substances of the serum, which when calculated to the corresponding amount of the original serum will be 0.5 c.c. of a 5% dilution.

Another alcohol insoluble fraction, globulin, has rather the action to inhibit the hemagglutination. This together with the hemolysis inhibitory principle of the albumin, is weakened by heating at 65°C. or above.

*Mechanisms of the Combination of the Inhibitory
Substances with the Toxin and Erythrocytes*

In order to determine what would be the mechanisms which play when the hemolysis inhibitory albumin fraction and the agglutination inhibitory globulin fraction would combine with toxin or erythrocytes, I have carried out the following experiments. The erythrocytes, the hemolysis and hemagglutination inhibitory substances and the toxin were mixed in vitro after various methods. The case in which the hemolysis inhibitory substance and the toxin had been first mixed and incubated at 37°C. for one half hour and then the erythrocytes had been added, gave the strongest hemolysis inhibition but if the erythrocytes and the inhibitory substance had been mixed and then the toxin had been added or if the toxin and the erythrocytes had been mixed, the inhibition was weaker. The hemagglutination, however, developed quite indifferently if the mixture should be made after whatever method of mixing. These results would seem almost contradictory to what is obtained by the

use of the serum instead of its components. They had a quite different physico-chemical nature from the original serum. They were hardly soluble in the saline and, therefore, if the solution of these substance should be left standing for a certain period of time or should it be centrifugalized, the inhibitory substances would be brought down and the supernatant fluid would contain only little of these substances. If, however, the precipitated fluid should be left standing for 24 hours or above, a large portion of the hemolysis inhibitory substance would again be dissolved into the saline. By making use of this nature, I carried out two sorts of absorption experiments and learnt some interesting facts.

(1) Combination Test of Toxin and the Inhibitory Substances: An equal amount of a 2% solution of the inhibitory substances, or alcohol insoluble fraction and a 1/200 solution of the toxin, which had been proved to contain twice as much threshold amount of the hemolysis inhibitory substance against one unit of hemolytic toxin. The mixture was incubated at 37°C. for 1/2-1 hour. It was then centrifugalized to obtain the large portion of both the hemolysis inhibitory and the hemagglutination inhibitory substances. The actions of the supernatant fluid and the saline emulsion of the precipitates upon the erythrocytes and the hemotoxin had been tested and the following results were obtained:

Tabele III

Preliminary treatment	Secondary treatment	Hemolysis	Hemagglutination
1 unit of hemolysis plus inhibitory substance (heated at 37°C. for 1/2 hour and then centrifugalized)	Erythrocytes mixed with the supernatant fluid (heated at 37°C for 1 hour)	Trace	Very strong
ditto	do. and toxin	Very strong	Very strong
ditto	Erythrocytes mixed with the precipitates	Trace	Trace
ditto	ditto and toxin	Strong	Weak
Control 1	Erythrocytes mixed with toxin	Very strong	Very strong
Control 2	Erythrocytes mixed with inhibitory substance	Trace	Weak

There occurred very weak hemolysis but a very strong hemag-

glutination when the supernatant fluid and the erythrocytes had been mixed. When the supernatant fluid, erythrocytes and toxin had been mixed, it gave very strong reactions to both hemolysis and hemagglutination. The mixture of both the erythrocytes and the precipitates gave a very weak if not practically a negative reaction to hemagglutination and hemolysis, while the mixture of the erythrocytes, precipitates and toxin gave a positive reaction to hemolysis and a very weak positive reaction to hemagglutination.

Judging from these experimental fact, it may be considered that the larger part of the hemolytic toxin would combine with the hemolysis inhibitory substance and is brought down into the precipitates, but hemagglutinin does not combine with the inhibitory substances. The large portion, however, would remain dissolved in the supernatant fluid. Moreover, the hemagglutination inhibitory substance would sediment by itself. It has been regretted that hemolysin could not be recovered from the sediments which consists of a combination of inhibitory substance and hemolysin.

(2) Combination of the Erythrocytes and Inhibitory Substance: The inhibitory substances which were employed in this experiment consisted of a 2% saline solution, which had been left standing for 24 hours from the dissolution. The solution was then mixed with the erythrocytes and left standing in the incubator at 37°C. for one half hour, when it was centrifugalized. The erythrocytes here obtained was washed several times. The influences of the hemotoxin and of the supernatant fluid upon the treated erythrocytes were tested and the following results were obtained:

Table IV

Experiment	Hemolysis	Hemagglutination	Notices
One unit of hemolytic toxin and guinea-pig erythrocytes sensitized with inhibitory substance	Strong	Weak	Hemolysis inhibition: weak Hemagglutination inhibition: strong.
One unit of hemolytic toxin, supernatant fluid and non-sensitized guinea-pig erythrocytes	Weak	Very strong	Hemolysis inhibition: strong Hemagglutination inhibition: practically none
Control 1: One unit of hemolytic toxin and non-sensitized guinea-pig erythrocytes	Very strong	Very strong	
Control 2: One unit of hemolytic toxin, non-sensitized erythrocytes and inhibitory substance	Weak	Weak	

The sensitized erythrocytes produced a very weak hemagglutination, but a strong hemolysis. The inhibitory substances were found not absorbed by the erythrocytes but remained free in the supernatant fluid. This might be explained on the ground that the hemolysis inhibitory substances have a very weak affinity to the erythrocytes, but the hemagglutination inhibitory substance a very strong affinity.

From the above described experimental facts, it might be justified to conclude that the hemolysis inhibitory substances would act directly against the hemolytic toxin, while the hemagglutination inhibitory substance first act upon the erythrocytes—presumably the receptor, which has a strong affinity to hemagglutinin—and then inhibitorily against the hemagglutinin.

Anti-body Formation

By immunizing the rabbit with either fresh or formalin treated sarcosporidiotoxin, a specific antitoxin had been obtained. It produced positive results in complement fixation and precipitation tests. The antibody formation by the hemotoxin, however, has not been dealt with. I have prepared fresh, formalin treated and heated hemotoxin. They were either singly or after being mixed with the pig's serum as Sachs proposed in the production of immune bodies by alcoholic extracts of lipoids, forcibly injected into the rabbits. The results were as follows:

(1) The rabbit was immunized with fresh toxin by 14 injections and obtained the serum which neutralized 200 times as large as the lethal dosis. The serum gave a positive complement fixation test up to $1/160$ and precipitation test up to $1/250\,000$. This serum, however, gave almost the same results as the normal serum in hemolytic and hemagglutinative tests, without giving any evidence of specific antibody formation for both of these reactions.

The immunized serum, which had been treated with either formalin or heat, also gave the negative results as the fresh one.

(2) The Antibody Formation of the Mixture of the Alcoholic Extracts and the Pig Serum: The rabbit was injected intravenously with a dosis of 3.0 c.c. of a mixture of the alcoholic extracts and the pig serum. 10-14 injections were given in all and

the serum was obtained from the blood withdrawn a week after the last injection. The immune serum gave positive results to the complement fixation and precipitation test, but negative to antihemolytic and antihemagglutinative reactions.

*Antitoxic Action of the Serum of the Rabbit Immunized
with the Alcoholic Extracts of the Toxin*

It was interesting to note that although the immune serum with the mixture of the alcoholic extracts of the toxin and the pig serum, as well as the one prepared with the alcoholic extracts alone, gave a negative result in antihemagglutinative and antihemolytic reactions in vitro, but it neutralized the toxic principle in the animals, which were susceptible to the sarcosporidiotoxin. By further experiments, it was found that 0.0005 c.c. (several times as large as the lethal dosis) of the toxin could be completely neutralized by 0.05–0.5 c.c. of the immune serum. From these experimental results, it would seem very probable that a certain portion of the sarcosporidiotoxin might be freed into alcohol in a state of so-called anatoxin, when it had been treated with a 90–95% alcohol.

RÉSUMÉ and CONCLUSION

1. Sarcosporidiotoxin contains hemolytic and hemagglutinative principles.
2. The optimal concentration of the erythrocytal suspension to test both of these reactions is 2.5–5.0%.
3. The physico-chemical characteristics of these two principles of the toxin: Hemolytic principle is hardly soluble in water, saline and glycerinated saline, but the hemagglutinative principle is soluble in these media; the one is soluble in alcohol and ether, but the other insoluble; The one is thermostable, but the other is destroyed by heating at 85°C. for 1/2 hour; The one is negative to protein reaction test, but the other positive; Concerning their resistances against acid and alkali, dialysis and adsorption, both of them almost agreed with each other.
4. The normal serum acts indifferently up to a certain amount but above this limit, it rather inhibits hemolysis by the hemolytic

principle of the Sarcosporidiotoxin.

5. Alcohol soluble fraction of the serum (or lecithin fraction) accelerates hemolysis by this toxin, but the insoluble fraction (or albumin fraction) and globulin fraction inhibit hemagglutinative action of the toxin.

6. It might be justifiable to conclude from the experimental data that the hemolysis inhibitory substance (albumin fraction) acts directly against hemolytic principle while the hemagglutination inhibitory substance (globulin fraction) first combines with the erythrocytes and consequently inhibits hemagglutination.

7. All the batches of the immune sera prepared with the sarcosporidiotoxin treated by divergent methods were indifferent to hemagglutination.

8. The serum of the rabbit immunized with the alcoholic extracts of the sarcosporidiotoxin, either simple or in mixture with the pig's serum, was indifferent in hemagglutination and hemolysis, but it neutralized the toxic principle.

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住肉胞子蟲血球毒ニ就テ

住肉胞子蟲毒素研究其二

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(昭和4年10月16日受附)

住肉胞子蟲毒素中ニ存在スル血球毒ノ理化學的及血清學的性質ハ從來一般

ニ知ラレタル動物性及植物性血球毒ニ對比シ大ニ趣ヲ異シ特ニ溶血毒ノ如キハ浸出法ニ周到ナル注意ヲ拂ハザレバ之ガ證明困難ニシテ更ニ本血球毒ニ對スル血清並ビニ血清各成分就中れちゝん、これすてりん及血清蛋白質等ノ催進作用又ハ阻止作用ニ於テモ蛇毒ノ如キ動物性血球毒トハ異同尠カラザルモノアリ次ニ其要點ヲ抄録スベシ

1. 住肉胞子蟲毒素中ニハ2種ノ血球毒即チ溶血毒及血球凝集素存在シ毒單獨ニテ洗滌血球ニ對シテ溶血作用及血球凝集作用ヲ發呈ス

2. 溶血作用 14種ノ可檢血球中海獺血球ガ最モ鋭敏ニシテ(溶血價6400倍)馬、家兎、犬、豚、人、山羊血球之ニ亞ギ(3200—1600倍)水牛、鵝、鳩、家鴨、緬羊、牛及黃牛血球ハ弱度ノ溶血反應ヲ呈ス(400倍内外)

3. 血球凝集作用 水牛血球ハ最モ高キ凝集價ヲ示シ(200000倍)人、家鴨及家兎血球ハ次ニ位シ(100,000倍)犬、鳩、馬、海獺、緬羊、牛、黃牛血球ト順次凝集價低下ス(50000—10000倍)

4. 兩種血球毒ノ反應ヲ檢測スルニハ2.5—5%ノ血球浮游液適度ナリ血球濃度之ヨリ稀薄ナル時ハ過剩阻止現象ノ圈域擴大シ且ツ血球ハ縱令溶血スルモ游離もくろびん沈澱シテ固有ノ色彩消失スルガ爲メ成績ノ鑑識困難ナリ

5. 血球毒ノ溶血作用及血球凝集作用ハ血球種類ニヨリテ其程度ニ著シキ相違アレドモ血球毒ト血球又ハ血球綱材トノ結合力(或ハ吸收力)ハ血球種類トハ無關係ナリ

6. 兩種血球毒ノ血球ニ對スル反應速度ハ相均シカラズ即チ血球凝集素ハ比較的迅速ニシテ血球ニ作用後數分間ニシテ現ハレ約2時間ニシテ頂點ニ達スルモ溶血毒ハ30分頃ヨリ徐々ニ反應現ハレ約20時間ニシテ完成ス

7. 血球毒ノ理化學的性狀 兩種血球毒ハ溶解性及耐熱性ニ於テ著シキ相違アリ即チ溶血毒ハあるこゝる及あるこゝるニ可溶性ナレドモ生理的食鹽水及ぐりせりん食鹽水ニハ難溶性(恐ラクコロイド狀ニ浮游シ來ルモノナルベシ)ナルヲ以テ抽出法特ニ磨碎法ヲ丁寧ニスベシ而シテ本溶血毒ハ耐熱性ナレドモ65° 30分以上ノ加熱ニヨリテ大部分沈澱物中ニ移行ス血球凝集素ハあるこゝる及あるこゝるニ不溶性ニシテ水及生理的食鹽水等ニ可溶性ニシテ85° 30分ニテ全く破壊セラル耐酸性滲透性及被吸着性等ニ就テハ兩種血球毒ハ略相均

シク共ニ酸ニ比較的強クあるかりニ弱クかおりん及動物炭ニヨリテ吸着セラレ動物膜ヲ通過セズ

8. 血球毒ニ及ス血清ノ作用 健康血清ハ少量ナレバ溶血毒ニ殆ト影響ナキモ一定量以上ヲ混加スレバ溶血作用ヲ全然阻止スルニ至ル該溶血阻止作用ハ同名血清ニテモ異種血清ニテモ大差ナシ而シテ 65°—80° 度加熱血清ハ溶血阻止作用稍減弱スルモ 100° 加熱血球ハ却ツテ新鮮血球ト殆ト同程度ノ阻止作用ヲ呈ス

血球凝集素ニ對スル血清ノ作用ハ混加ノ順序ニヨリテ多少相違シ前ニ毒ト血清トヲ作用シ後血球ヲ加フル時ハ凝集價ハ一般ニ著明ノ減弱ヲ來ス

9. 血清各成分ノ血球毒ニ對スル作用 血清ノあるこ一る可溶成分ハ溶血作用ヲ催進シ不溶成分ハ本血球毒ノ溶血作用及血球凝集作用ヲ阻害ス

(イ) 溶血催進物質ノ主體ハれちゝん・ふらくちよんニシテ中性脂肪ハ弱度ノ催進作用ヲ有シけふゐりん及くゝーりんハ本溶血毒ニ對シテ催進作用ヲ呈セズ

(ロ) これすてりんハ本溶血毒ニ對シテ殆ト何等ノ影響ヲ呈セス

(ハ) 溶血阻止作用ノ主體ハあるぶみん・ふらくちよんニシテぐろぶりん・ふらくちよんハ血球凝集阻止作用ヲ呈ス

10. 結合又ハ吸收試験ノ結果ヨリシテ溶血阻止物質ハ直接ニ溶血毒ニ結合シテ溶血阻止作用ヲ呈シ血球凝集阻止物質ハ先ツ血球ニ結合シ二次的ニ血球凝集阻止作用ヲ呈スルモノト考ヘラル

11. 本血球毒ニハ抗原性ヲ認ムルコト能ハズ即チ新鮮毒ふゐるまりん處置毒免疫血清(共ニ毒主成分ニ對シテ抗毒素作用ヲ有ス)及加熱毒免疫血清並ニ本毒素ノあるこ一る浸出物ニ豚血清ヲ加ヘテ反覆家兎ニ注射(10—16回)シテ得タル免疫血清モ抗溶血體及抗血球凝集素ヲ證明スルコト能ハズ但シあるこ一る浸出毒免疫血清ハ豚血清ヲ混加スルト否トニ拘ラズ住肉孢子蟲毒素ノ毒主成分ニ對シテ抗毒素作用ヲ有ス