

ら推察すると、血管変性は血管周囲の炎症反応に基づく変化であることも考えられた。

### <資料 13>

#### カ) 狂犬病における応用

本病はラブドウイルスにより起こる人畜共通伝染病で、タイにおいて重要な疾病の一つである。

本病の診断法は、脳アンモン角部の押捺標本における蛍光抗体法及びマウス接種法である。

今回、狂犬病にABC法を応用する目的は診断へのアプローチではない。

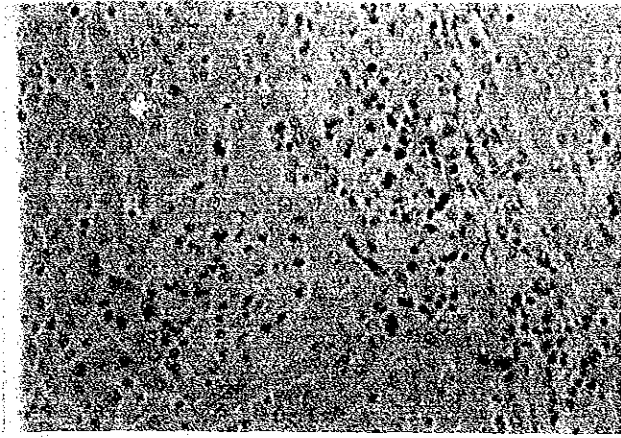
一般に、狂犬病ウイルス株は一国一種と考えられていたが、マダガスカル、タイ、イランにおいて、流行ウイルス株の異なることを示唆する報告 (American Journal of epidemiology, vol 117) があることから、モノクロナール抗体を使用したABC法でウイルスstrainを検索・分析することが可能であるか試験を行った。

パラフィン切片 (野外発生例) を用い、抗血清濃度、作用時間等種々工夫したABC法を行ったが、特異的陽性所見を得ることはできなかった。

モノクロナール抗体の場合、ポリクロナール抗体を使用した場合に比較して、抗原抗体反応が弱いことが十分に考えられる。また、抗原性の賦活化、抗血清希釈濃度、非特異反応の除去等種々検討することが必要と考えられた。

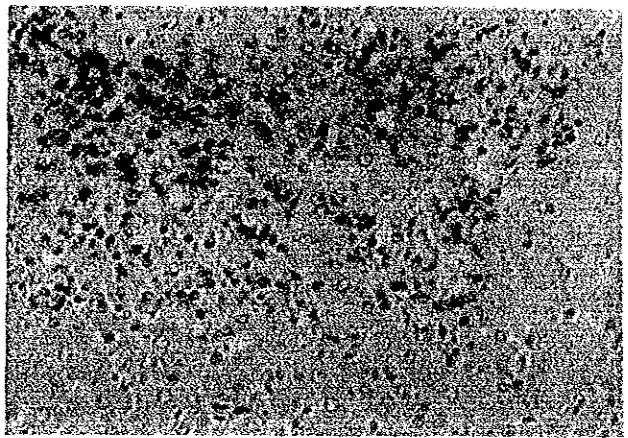
なお、ウイルスストレインの解析について、DLCウイルスセクションにおいて間接畜光抗体法により、その検索を進めてもらうよう依頼した。





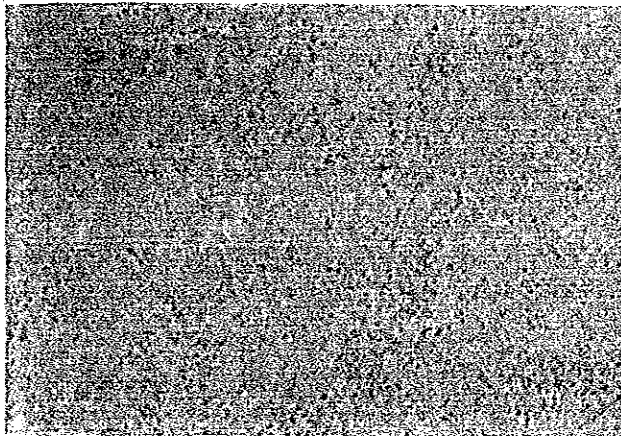
Picture1: Swine, liver, H-E stain.

巣状壊死が多発しており、グリソン氏鞘に細胞浸潤を認める。類洞MPSの活性化も著しい。これら部位にターミナルコロニーが多数認められる。



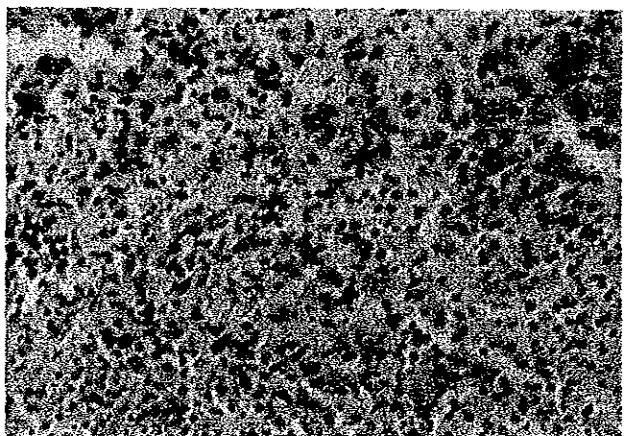
Picture2: Swine, liver, ABC stain.

ターミナルコロニーの他、類洞マクロファージ、浸潤マクロファージ内に、単在する原虫も容易に検出される。非特異反応は除去されている。



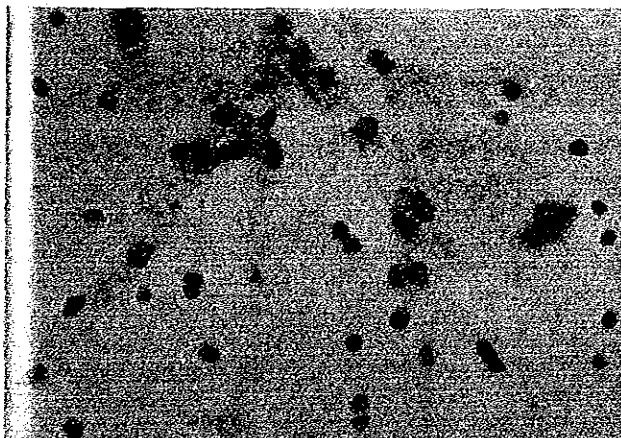
Picture3: Swine, liver, ABC stain with antibody-negative serum.

内因性ペルオキシダーゼの反応はみられない。



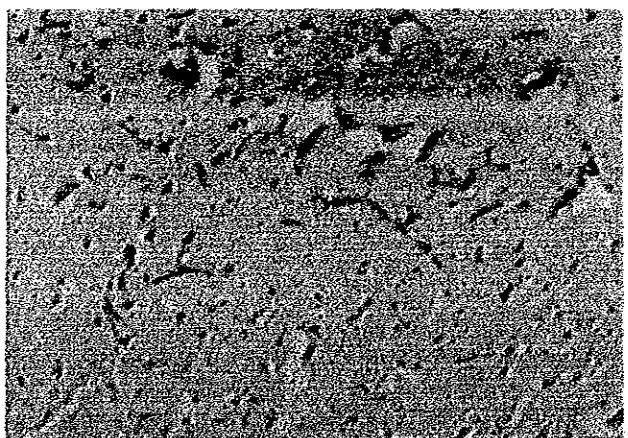
Picture4: Swine, liver, ABC stain.

巣状壊死の多発がみられるが、ターミナルコロニーの形成は少なく、単在する原虫が認められる。しかし、非特異反応が強いため、再検討を必要とする。



Picture5: Bovine, brain, H-E stain.

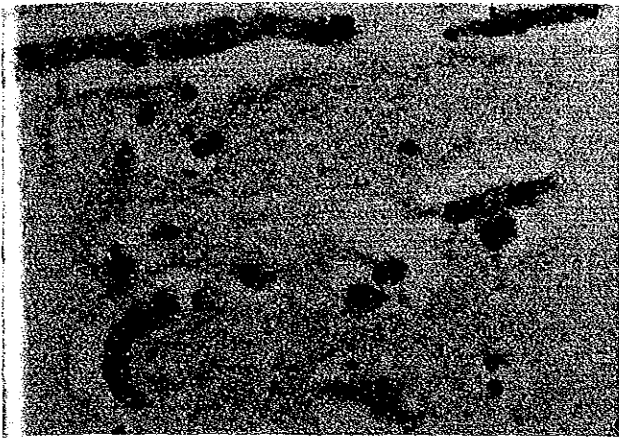
脳毛細血管内に Babesia bovis の寄生する赤血球が停滞している。血管内皮細胞の腫大と血管周囲性の浮腫が著名に認められる。



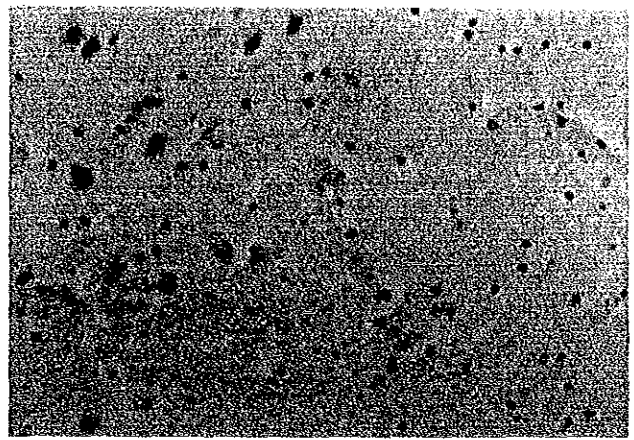
Picture6: Bovine, brain, ABC stain.

H-E染色に比べ、毛細血管内の原虫寄生赤血球が明瞭に認められ、脳内における分布を容易に把握できる。

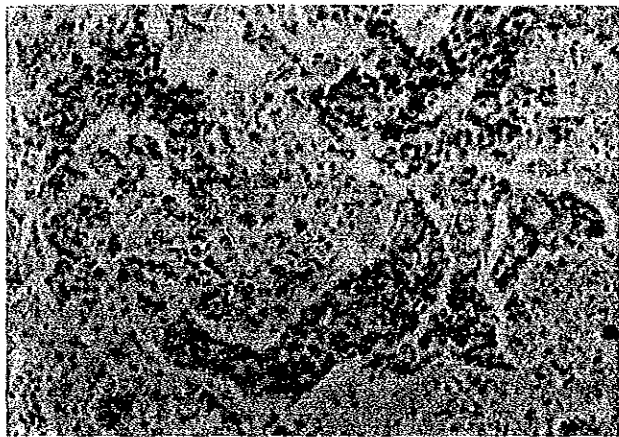




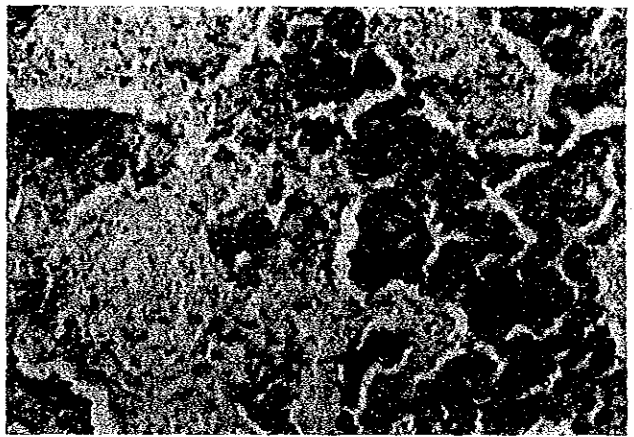
Picture7 : Bovine, brain, ABC stain  
原虫及び寄生赤血球が染められているほか、血管外に少数の陽性顆粒状が散見される。



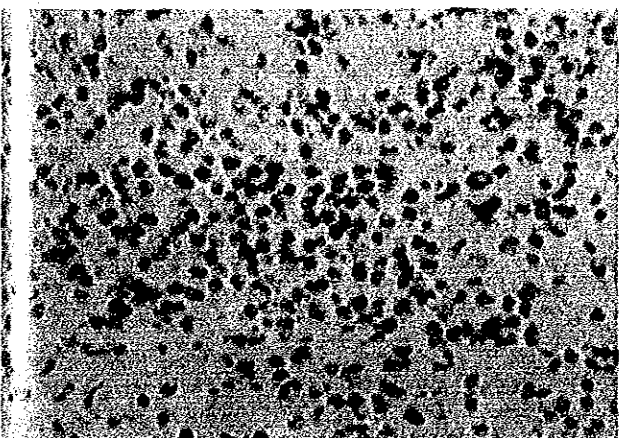
Picture8 : Bovine, brain, ABC stain with antibody-negative serum.  
毛細血管内赤血球がわずかに染色されているが、陽性例と比べると明瞭に識別される。



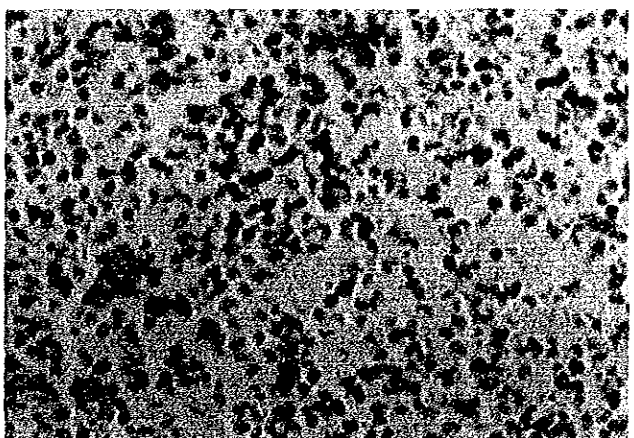
Picture9 : Swine, lung, ABC stain with antibody-negative serum.  
肺胸腔に線維芽細胞様の細胞増殖が著明に認められる（肉芽様病変の形成）。中央部にヘマトキシリンに弱く染まる菌塊がみられ、出血性傾向が強い。



Picture10 : Swine, lung, ABC stain  
肉芽用病変部に一致して陽性所見を認める。菌分布は気道肺胞領域が主で、間質における菌分布は少ない。



Picture11 : Swine, lung, ABC stain.  
肺胸膜に著しい線維素の析出と細胞浸潤が認められた症例で、写真は胸膜面に認められた陽性所見である。H. parasuis は滲出細胞に貪喰されているものが多く、線維素部分にも少数の陽性菌が散見される。



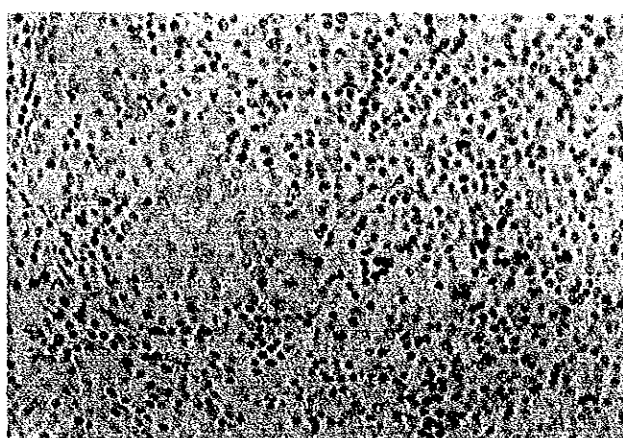
Picture12 : Swine, lung, ABC stain with antibody-negative serum.  
picture11と同様部位であるが陽性所見は認められない。





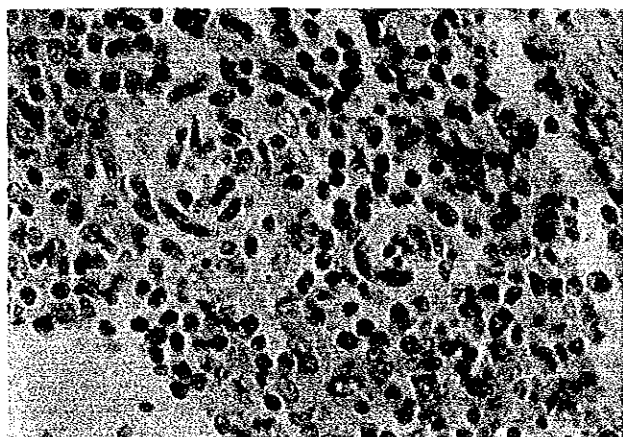
Picture 13 : Swine, tonsil, ABC stain.

扁桃粘膜上皮細胞が陽性に染色されている。粘膜下結合線維に弱陽性反応がみられる。リンパ組織は疎性化している。



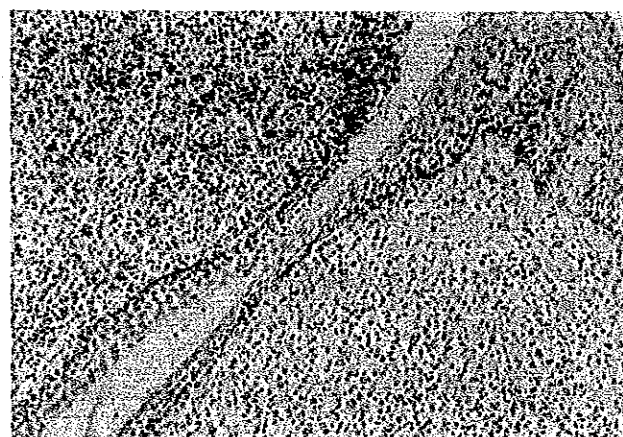
Picture 14 : Swine, tonsil, ABC stain.

扁桃リンパ組織二次小節のリンパ球減少と同部位における細網様細胞の陽性所見。



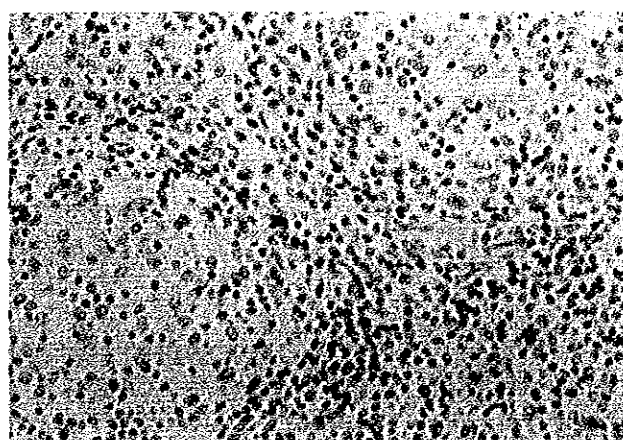
Picture 15 : Swine, spleen, ABC stain.

血管周囲リンパ組織における細網様細胞の陽性所見。血管壁は浮腫性であるが内皮に陽性所見は認められない。



Picture 16 : Swine, tonsil, ABC stain with antibody-negative serum.

粘膜上皮細胞に陽性所見は認められない。



Picture 17 : Swine, tonsil, ABC stain with antibody-negative serum.

扁桃リンパ組織二次小節において陽性所見は認められない。





### T i t e r a t i o n o f a n t i s e r u m s

When We search for proper titer of antiserum ,  
titeration of antiserum ( as below )

×16 ×32 ×64 ×128 ×256 ×512 ×1024 ×2048 ×4096 ×8192

or

×10 ×20 ×40 ×80 ×160 ×320 ×640 ×1280 ×2560 ×5120 ×10240 , Search  
for proper titer by use underline titer serums .

### R e s u l t

Toxoplasmosis	proper titer	×512~×1024 (rabbit serum)
Babesia bovis	proper titer	×400 (bovine serum)
Actinobacillus		
pleuropneumoniae	proper titer	×4096 (rabbit serum)
Haemophilus		
parasuis	proper titer	×4096 (rabbit serum)
Swine Fever	proper titer	×400~×800 (rabbit serum)
Rabies	proper titer	? (mouse serum)

## Histopathological Mechanism of Inflammation

Inflammation is defined the reaction of a living body against Injurious stimulus .

### Cause of Inflammation

Animate matters : Bacteria , Rickettsia , Virus , Fungi , Parasite , others .

Inanimate matters: Physical Injurious Action , Heat and Cold , Powder dust , others .

### Histological change

Reaction of blood vessel : Hyperemia and Exudation in System of micro circulation .

Increasing of cells : Macrophage , Lymphocyte , Fibroblast

### 1 Reaction of blood vessel

#### (1) Inflammatory exudation stage

< Exasperation of blood vessel permeation-ability >

First stage : for short term

- \* be concerned in Histamine , Serotonin , other Amines .
- \* Mast cell release amines and PAF by connection with IgE = Degranule
- Mast cell have Fc-receptor of IgE .
- \* PAF = Platelet Activate Factor

Secondary stage : Durable reaction

- \* Participation of Macrophage in tissue
- Macrophage make thiolprotease (SH-protease) that exasperate of vessel permeate ability , by phagocytization of inflammatory cause .
- \* Participation of Neutrophils and Macrophage
- Arachidonic acid metabolite ( Prostaglandin , Leuko-

trienes ) exasperate permeate-ability of vessel , in the process of Ingestion and Digestion ( Phagocytization ) .

\* Participation of Complement

Serine-protease that is derived from neutrophils disintegrate C3 and C5 .( to C3a,C3b and C5a,C5b ) .

C3a and C5a are anaphylatoxin , act for exasperation of vessel-permeate-ability .

And C3a , C5a have Activate-factor of Emigration .

C5b connect with target cell , and induce connection with C6,7,8,9 , then kill bacteria .

C3b connect with C3-Receptor of phagocyte , and be exhibited Opsonisation .

(2) Infiltration of neutrophilic leukocyte (Chemotaxis )

SH-protease that is derived from Macrophage act to IgG in tissue (exudated from serum ) , as a result , leukotaxine be made up .

and others , Neutrophils exhaust leukotoriene system matter.

2 Infiltration and Increasing of Macrophage , Lymphocyte and Connective tissue system cells

Infiltration of Macrophage is related to MCF ( Macrophage Chemotactic Factor )

MCF-a : Formation due to < Serineprotease is derived from neutrophils act to IgG > .

MCF-b : Formation due to < Serineprotease is derived from neutrophils act to Complement5 > .

MCF-c : Lymphokine .

MCF-a,b is detected a large quantity in local position of immediate type hypersensitivity .

MCF-c is detected a large quantity in local position of delayed type hypersensitivity .

Infiltration of Lymphocyte be related to LCF ( Lymphocyte Chemotactic Factor ) .

Unkown in details , maybe SH-protease that is derived from Macrophage act to IgM , IgG .

LCF that is detected in local position of derayed type hypersensitivity have 4 kinds of factor .

Factor a : act to B-cell , don't act to T-cell

Factor b,c,d : act to T-cell , don't act to B-cell

Subsequent Inflammatory reaction develop depend on Lymphokines

Increasing of Connective tissue system cells

- Fibroblast , Macrophage , Capillary e.t.c -

I L - 1 : activate Fibroblast .

be produced by Macrophage .

Lymphokine : act to Macrophage and Capillary .

表1 おもなサイトカン

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リンパ球由来 (リンフォカイン)
インターロイキン2 (IL-2)
インターロイキン3 (IL-3)
インターロイキン4 (IL-4, BSF-1)
インターロイキン5 (IL-5)
B細胞刺激因子-2 (BSF-2) { L-6
B細胞増殖因子 (BCGF)
ガンマー・インターフェロン (IFN $\gamma$ )
リンフォトキシン (LT)
顆粒球マクロファージ・コロニー刺激因子 (GM-CSF)
マクロファージ遊走阻止因子 (MIF)
マクロファージ由来 (モノカイン)
インターロイキン1 (IL-1)
腫瘍壊死因子 (TNF)
アルファ・インターフェロン (IFN $\alpha$ )
顆粒球・コロニー刺激因子 (G-CSF)
他の細胞由来
ベータ・インターフェロン (IFN $\beta$ )
マクロファージ・コロニー刺激因子 (M-CSF)

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サイトカイン全般に共通する性状

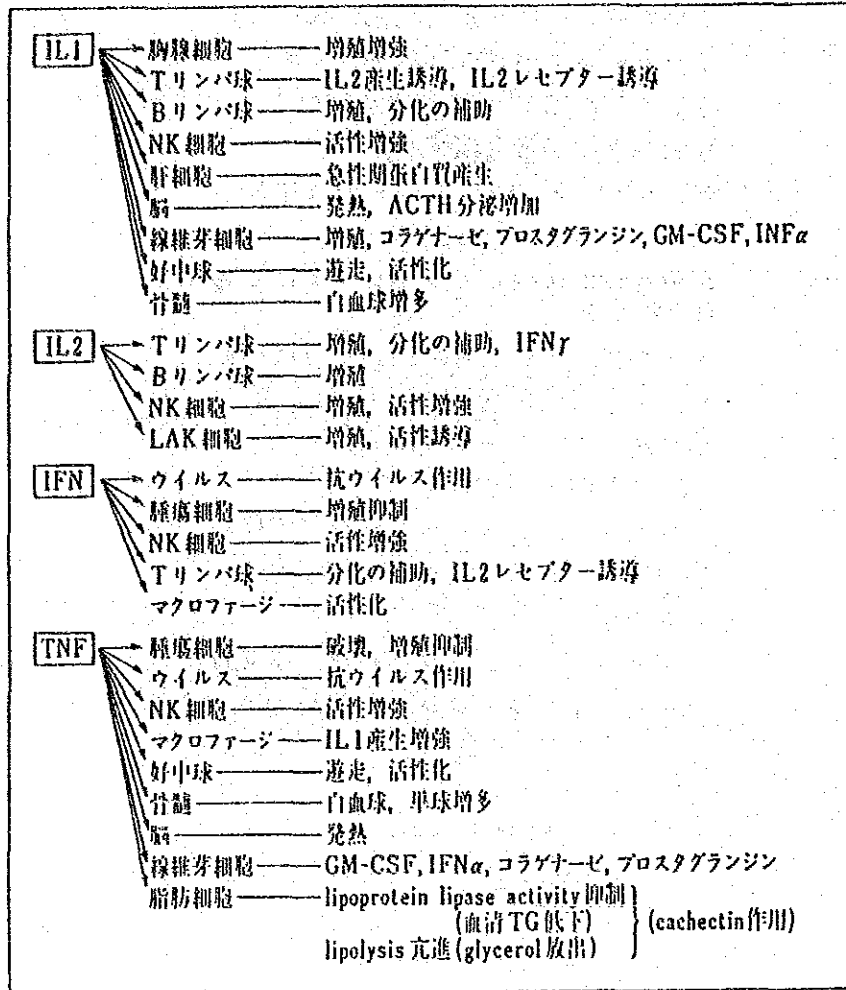


図1 サイトカインの多様な生物活性

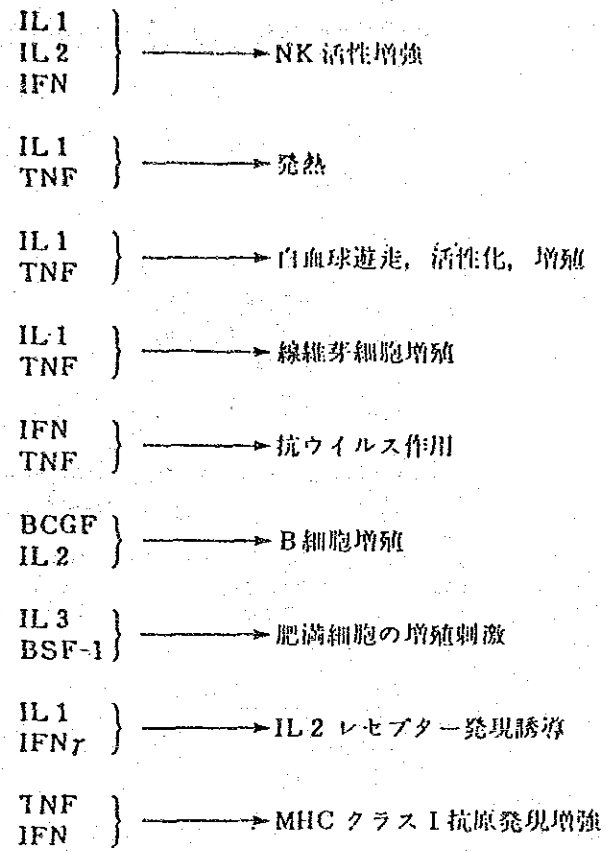


図 2. 異なるサイトカインが同じ作用を示す例

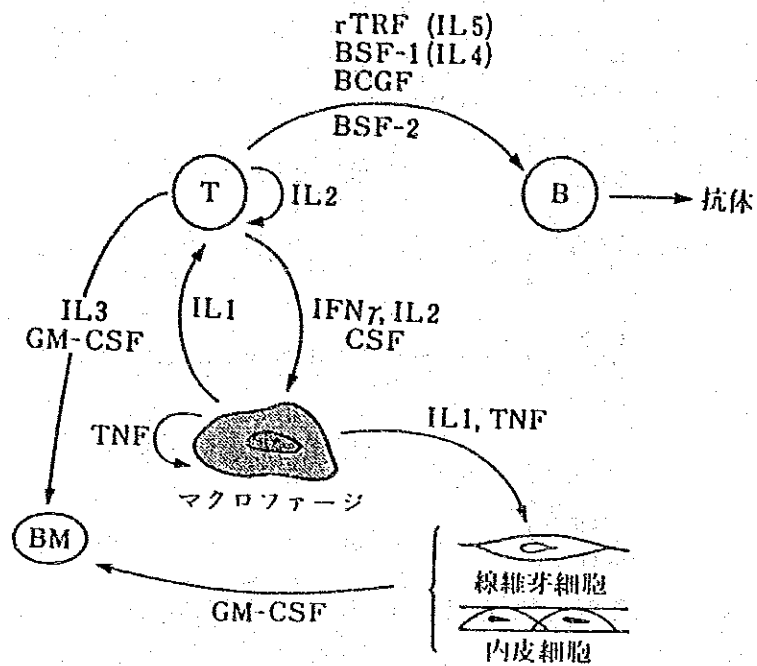


図3 サイトカイン産生における各種サイトカインの相互依存性



Mr. Vitoon Kamnerdpeth  
Director General  
Department of Livestock Development  
Ministry of Agriculture and Cooperative  
Phayathai Road , Bangkok  
Thailand

Dear Sir ,

It is a great pleasure for me to submit the report of my assignment carried out from July 25th to October 24th on after-care program of the Animal Health Improvement Program of South Regional Veterinary Research and Diagnostic Laboratory Center , Thung Song .

I would like to take this opportunity to express my gratitude for the kind collaboration and hospitalities of your staff of center at Thung Song .

I am leaving for Japan at October 24th , but from this time forth , I will retain close contact with Thai colleagues for future development of research and diagnosis for animal diseases .

Your sincerely ,

Masakatsu Kato

The main purpose of my duty is to transfer the technique of Immunohistochemical examination to the counterpart, Dr. Pipol Sukaithaichana, and to apply this method to some infectious diseases, during the period in The South Regional Veterinary Research and Diagnostic Laboratory Center. The other duties are to transfer my technique and knowledge about the pathological diagnosis of animal diseases and how to investigate the diseases.

In Immunohistochemical technique, some methods are known.

The method that I transferred in this time is Immunoperoxidase technique, especially Avidin-Biotin peroxidase Complex method (the following: ABC method).

Immunoperoxidase technique can prove the localization of antigen (for example, pathogenic organ and tumor-cell's marker, immunoglobulin etc) that exist in tissue or cell, by using antigen-antibody reaction.

ABC method is the most sensitive in immunoperoxidase techniques.

I applied this method to some infectious diseases. My purpose isn't to establish as diagnostic method, but is to investigate relation between antigen and histopathological changes.

The diseases applied with ABC method are Toxoplasmosis, Babesia bovis infection, Actinobacillus pleuropneumoniae infection, Haemophilus parasuis infection, swine fever, rabies.

I believe that this ABC method was transferred to counterpart, by repeating it for some infectious diseases.

#### 1. The outline of ABC method

The outline of the principle, the technique and the attention points of ABC method are described on the attached paper No 1~No 6.

## 2. Application of ABC method for some infectious diseases

### (1) Toxoplasmosis in swine

At present , the most reliable diagnostic method in this disease is confirmation of Toxoplasma , therefore Giemsa-stain and fluorescent-antibody-test are applied .

The histopathological investigation can also find out terminal-colony of toxoplasma in the focus , but in the case of few terminal-colony form and some singleform toxoplasma in the foci , the finding out toxoplasma often is difficult .

In such a case , ABC method is useful because this method is able to prove the localization of toxoplasma's antigen on the paraffin sections

#### - Result -

In the application for toxoplasmosis , we could confirm the reliable meaning of positive views . Because the size of toxoplasma is microscopic .

After this , we should try to except non-specific-reaction . Or else , the positive views will lose the reliable meaning .

### (2) Babesia bovis infection in bovine

Many of B.bovis infection in bovine is acutely fatal , before B. bovis increase in peripheral blood .

This is caused by the failure of gaseous metabolism due to adhesion and stagnation of infected erythrocytes in brain blood capillary .

In diagnosis , the investigation of brain's stamp smear is very important .

- Result -

The application of B. bovis is relatively easy , but it is necessary to confirm the specificity of antiserum . Because this antiserum contains anti-bovine erythrocyte-antibody . In this study , we obtained the good result . But after this , it is necessary to inquire further into the problem of specificity .

### (3) Actinobacillus pleuropneumoniae infection in swine

Once , this bacterium was included among genus Haemophilus , but now is classified as genus Actinobacillus .

A. pleuropneumoniae is very important as a cause of swine respiratory infectious diseases , often cause fibrinous-pleuropneumonia , and show high mortality .

Mycoplasma , Pasteurella , Actinobacillus , some viruses and so on are known as pathogens causing pneumonia .

I think that the histopathological investigation of the sections stained by specific antibody will serve a good reference to understand the respiratory disease's mechanism .

- Result -

The typical lesion in this case often was found out in the cases of Haemophilus or Pasteurella infection as well . The agreement in location of the lesion and bacteria is important to determine pathogen .

Though we tried to investigate some field cases from which Haemophilus spp were isolated , we couldn't obtain the positive cases .

In one case , we obtained the positive views , but this case was the mixed infection and the positively stained bacteria were of small number .

Generally , the respiratory disease seldom cause by single pathogen , the mixed infection is frequent . Therefore , it is necessary to apply ABC method against many pathogens for the investigation of the respiratory diseases .

(4) Haemophilus parasuis infection in swine

This bacterium is the causal agent of Glasser's disease .

In histopathological change , this disease show fibrinous serosa-arthritis . Besides , H. parainfluenzae cause Meningitis and Pleuropneumonia .

For Haemophilus infected cases in south district , we investigated the ability of pathogen-identification on paraffin sections .

- Result -

We examined some outbreak cases having Serositis ( mainly Meningitis ) with the purpose to identify H. parasuis .

But we couldn't obtained the positive views .

This result is not a complete denial of the participation of H. parasuis . Meningitis and pleuropneumonia are able to be caused by H. parainfluenzae etc . Therefore , we think that it is important to investigate the participation of other bacteria for understanding the relation between the histopathological changes and pathogens .

(5) Swine fever in swine.

This disease is very contagious and severe in symptoms , and show high mortality . Therefore , swine fever is the most important infectious disease in swine .

Direct fluorescent-antibody method of tonsil is most reliable in diagnosis

My purpose in application of ABC method isn't the approach for diagnostic method , is to investigate of the relation between the histopathological changes and antigen in swine fever .

- Result -

In the application to swine fever , we think that it is difficult to stain and to confirm the meaning of positive views in comparison with parasitic or bacterial diseases .

Because the viral antigens easily lose the activity in the process of making up paraffin section ( for example ; formalin , heat treatment ) .

Therefore , Activation of antigen by pronase treatment is very important .

We investigated the distribution of swine fever antigen .

As a result , swine fever virus antigen distributed in lymphatic tissue ( tonsil and spleen , lymphonode ) . Especially , we obtained the positive views in tonsil epithelium and RES cells of lymphatic tissues .

In the reading of the result , it is necessary to be cautious , because the examined case was a small number and couldn't completely avoid the non-specific-reaction .

#### (6) Rabies

This disease is caused by Lyssavirus , and is one of the important diseases in Thailand .

The diagnosis is made by the confirmation of Negri body by FA test .

In this study , ABC method and monoclonal antibodies were applied to differentiate study the virus-strains , because the report in [ American Journal of Epidemiology, vol117 ] described that some different rabies virus strains are prevailing in Thailand .

- Result -

We couldn't obtained the specific positive views on paraffin sections .

But , I think that the studies of virus-strain are very important in future control of rabies .

Therefore , I have trusted virology-section in S-DLC with cooperation of this study .

### 3. Other duties

#### (1) Immunological background in inflammation

I outlined on attached paper No 7~No 8 .

#### (2) Introduction of some diseases

Reference ;

- \* Pathogenesis of Johne's disease and its control

Journal of Japan Veterinary Medical Association Vol.42 No 4

1989 .

- \* Evaluation of autofluorescence as an aid to diagnosis of  
cerebro-cortical-necrosis

The Veterinary Record , October 9 , 1982 .

- \* Congenital cerebellar hypoplasia in newborn calves

Nat,Inst,Anim, Hlth Quart , 19 , 1979 .







JICA