BIKEN'S JOURNAL 2, 27-29 (1959)

The Immunological Relationship of the Poxvirus Group

Immunological studies of the pox group viruses have been made by Downie, Burnet, Fenner etc. (1956, 1957), and it has been shown that ectromelia, vaccinia, variola and cowpox viruses have an immunological relationship in neutralization, complement fixation and hemagglutination. The immunological relationship between vaccinia and myxoma viruses has not been reported as yet. Fowlpox has been described to be unrelated to the other pox viruses.

Recently Kamahora, Kato and their colleagues (1955, 1955, 1958, 1959b) have reported that ectromelia, fowlpox, vaccinia and cowpox viruses form two kinds of inclusion body namely "A" and "B". Of these, the "B" type inclusion body has been found to have common morphological and cytochemical characteristics. It is Feulgen positive and stained reddish purple with Giemsa solution. More recently Kato and Cutting (1959a) have found that myxoma and fibroma viruses also formed "B" type inclusion bodies in infected cultured human amnion cells as well as in tumor cells. It has thus been surmised that all pox viruses form "B" type inclusion bodies of the same morphological and cytochemical nature.

Lately we (1958) have succeeded in demonstrating that the "B" type inclusion bodies of myxoma virus are composed of viral antigen. This was shown with fluorescein-labelled antibody, and it is assumed that this inclusion body might be the main site of virus multiplication.

This letter shows that a close immunological relationship exists among all pox viruses in the complement fixation reaction and that the "B" type inclusion bodies of all pox viruses have a common antigenic substance.

Myxoma immune rabbit antiserum obtained by repeated inoculation of a UV-irradiated myxoma tumor suspension was found to react with all other pox viruses (ectromelia, vaccinia, cowpox, fowlpox and fibroma) in the complement fixation reaction.

Table 1. Complement Fixation Reaction among Pox Group Viruses (I)

Antibody / Antigen	Myxoma Immune Rabbit Serum					
	20×	40×	80×	160×	320×	640×
Myxoma	###	###	###	###	##	−
Fibroma	###	###	###	##	+	−
Ectromelia	###	###	###	##	−	−
Vaccinia	###	###	###	##	−	−
Vaccinia (I. H. D.)	###	###	###	##	−	−
Cowpox	###	###	###	##	−	−
Fowlpox	###	###	##	+	−	−

Table 2. Complement Fixation Reaction among Pox Group Viruses (II)

Antigen \ Antibody	Vaccinia Immune Rabbit Serum					
	20×	40×	80×	160×	320×	640×
Myxoma	###	###	##	+	−	−
Fibroma	###	###	##	+	−	−
Ectromelia	###	###	###	##	+	−
Vaccinia	###	###	###	###	##	+
Vaccinia (I. H. D.)	###	###	###	###	##	+
Cowpox	###	###	###	###	##	+
Fowlpox	###	###	###	##	+	−

Vaccinia immune rabbit serum obtained by a single subcutaneous injection and additional intravenous injection of the virus material, also reacted with other pox group viruses. The results are shown in Table 1 and 2.

Thus it is shown that all pox group viruses have a close immunological relationship in complement fixing reaction.

The cytoimmunological relationship of the "B" type inclusion bodies of all pox viruses was examined by the fluorescent antibody method. When ectromelia infected Ehrlich tumor cells were stained with myxoma fluorescent antibody, the "B" type inclusion bodies had brilliant fluorescence as seen in Fig. 1 and 2. In the same way the "B" type inclusion body of vaccinia, fibroma and cowpox could be stained with the same myxoma fluorescein-labelled antibody.

Therefore almost all the "B" type inclusion bodies of the pox group viruses have common antigenic material.

The designation of pox group virus has hitherto been based mainly on similarity in size of the virus particles and the pathological changes caused in infected animals. The finding of the immunological crossing and of the common immuno-cytochemical attitudes of the "B" type inclusion bodies characterises the pox group viruses more specifically.

Details of the results will be published soon in this Journal.

REFERENCES

Downie, A. W. and Dumbell, K. R. (1956) Pox Viruses. *Ann. Review of Microbiol.*, **10,** 237-252.

Fenner, F. and Burnet, F. M. (1957) A short description of the poxvirus group (Vaccinia and related viruses). *Virology*, **4,** 305-314.

Kamahora, J., Kato, S., Baba, E. and Hagiwara, K. (1955) Studies on the inclusion bodies of fowlpox virus. *Med. J. Osaka Univ.*, **6,** 745-753.

Kamahora, J., Sato, Y., Kato, S. and Hagiwara, K. (1958) Inclusion body of vaccinia virus. *Proc. Soc. Exp. Biol. Med.*, **97,** 43-47.

Kato, S., Hagiwara, K. and Kamahora, J. (1955) The mechanism of the growth of ectromelia virus propagated in the ascites tumor cells. *Med. J. Osaka Uuiv.*, **6,** 39-50.

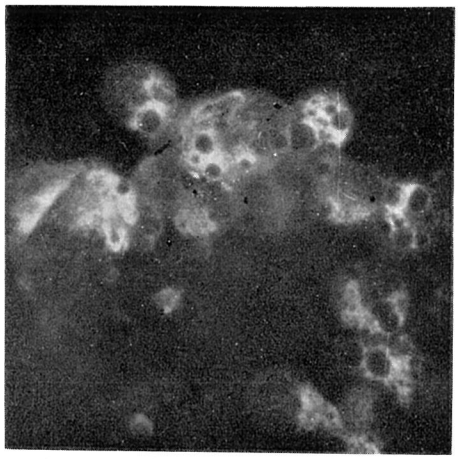

Fig. 1. The Ehrlich ascites tumor cells infected with ectromelia virus show brilliant fluorescence when treated with fluorescein-labelled antimyxoma rabbit serum.

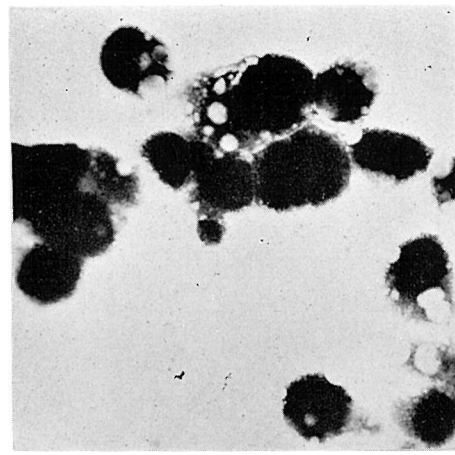

Fig. 2. The same field as Fig. 1. stained with Giemsa solution. All bright fluorescent areas correspond to "B" type inclusion bodies and dark round areas to "A" type inclusion bodies.

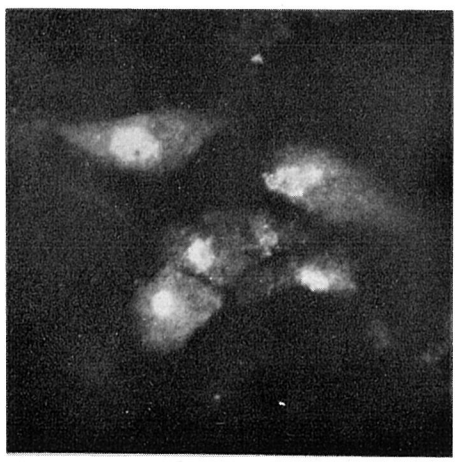

Fig. 3. The FL cells infected with vaccinia virus also show fluorescence within cytoplasm when treated with fluorescent antimyxoma serum.

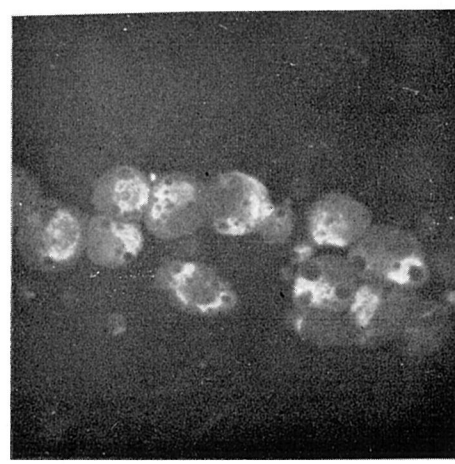

Fig. 4. The Ehrlich ascites tumor cells infected with cowpox virus. The "B" type inclusion bodies are stained with fluorescent antimyxoma serum while "A" type bodies remained unstained.

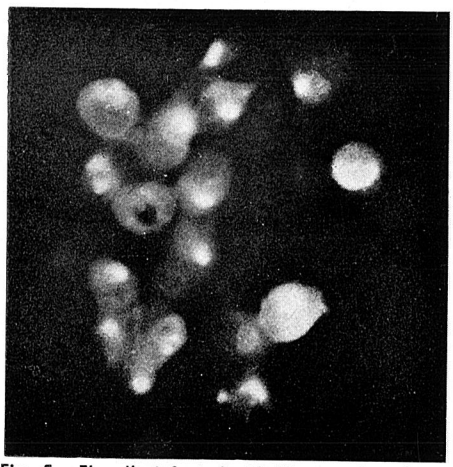

Fig. 5. FL cells infected with fibroma virus also show distinct fluorescence when stained with fluorescent antimyxoma serum.

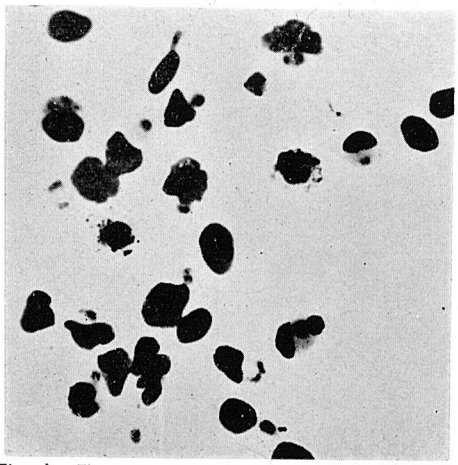

Fig. 6. The same field as Fig. 5. stained with Giemsa solution. Generality the stainability of cytoplamic RNA becomes very weak and the brilliant areas correspond to "B" type inclusion bodies.

Kato, S. and Cutting, W. (1959a) A study of the inclusion bodies of rabbit myxoma and fibroma virus and a consideration of the relationship between all pox virus inclusion bodies. *Stanford Med. Bull.*, **17**, 34-45.

Kato, S., Takahashi, M., Kameyama, S. and Kamahora, J. (1959b) A study on the new inclusion body of cowpox virus. *Bikens' J.* **2** (2) will be published.

Takahashi, M., Kameyama, S., Kato, S. and Kamahora, J. (1958) A study of myxoma virus inclusion with fluorescein-labeled antibody. *Biken's J.* **1**, 198-200.

MICHIAKI TAKAHASHI
SUSUMU KAMEYAMA
SHIRO KATO
JUNTARO KAMAHORA

Department of Pathology,
Research Institute for Microbial Diseases,
Osaka University.
Osaka Japan
Received May 20, 1959