BIKEN JOURNAL Vol. 11, 343-353, 1968

PRELIMINARY REPORT

IMMUNE HEMADSORPTION BY CELLS INFECTED WITH POXVIRUSES[1]

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(Received September 30, 1968)

The immune hemadsorption method was first applied by HÖGMAN (1959) to detect human blood group antigens on the surface of cultured cells. FAGRAEUS and ESPMARK (1961) applied this method to cultured cells infected with various viruses to demonstrate viral antigen on the surface of the infected cells. Positive results were obtained on cells infected with vaccinia, measles and canine distemper viruses.

This communication outlines some attempts to indicate viral antigens present on the cell surface of cultured RK_1 and FL cells infected with Shope fibroma virus, cowpox virus, vaccinia virus, and variola virus by the immune hemadsorption method and the fluorescent antibody technique.

The method for immune hemadsorption used is fundamentally similar to that of MILGROM *et al.* (1964). Shope fibroma virus (OA) was grown on a monolayer culture of RK_1 cells in Ham F12 with 10% calf serum and cowpox virus (LB red " A "v^-) on FL cells in Eagle's minimum essential medium with 5% calf serum. Viral antibodies were obtained from rabbits. Using the complement fixing test, the titers of the original rabbit anti-Shope fibroma virus serum and the original rabbit anti-cowpox virus serum were $512\times$ and $1{,}024\times$ respectively. Viral antiserum was absorbed with sheep red cells. Two parts of antiserum diluted 1:10, were mixed with one part of packed sheep red cells. Tubes were kept at room temperature for 30 min, and at 4°C for another 30 min. Then they were centrifuged at 3,000 rpm for 10 min and the supernatants was separated. Cells were grown on coverslips in Leighton tubes. There were 5×10^5 cells per tube. A half ml of cowpox virus (1.4×10^6 PFU/ml) and Shope fibroma virus with a virus titer which could produce almost 100% incidence of " B " type inclusion-bearing cells in infected RK_1 cells 24 hrs after infection, were inoculated into the each Leighton tube. Twenty four hours after viral inoculation, cultures were washed three times with Hanks' salt solution. After decanting the washing solution, 1.0 ml of various concentrations of antiviral rabbit serum were added to the cultures. Tubes were kept at 25°C for 1 hr. After washing out the antiserum with Hanks' salt solution, the attached antibody was detected by a complete indicator system consisting of sheep erythrocytes sensitized with

1 Presented at the Second Symposium of the Society of Japanese Virologists, Tokyo, September, 16, 1968

FIGURE 1 Positive immune hemadsorption on cowpox virus-infected FL cells.

FIGURE 2 Positive immune hemadsorption on Shope fibroma virus-infected RK_1 cells.

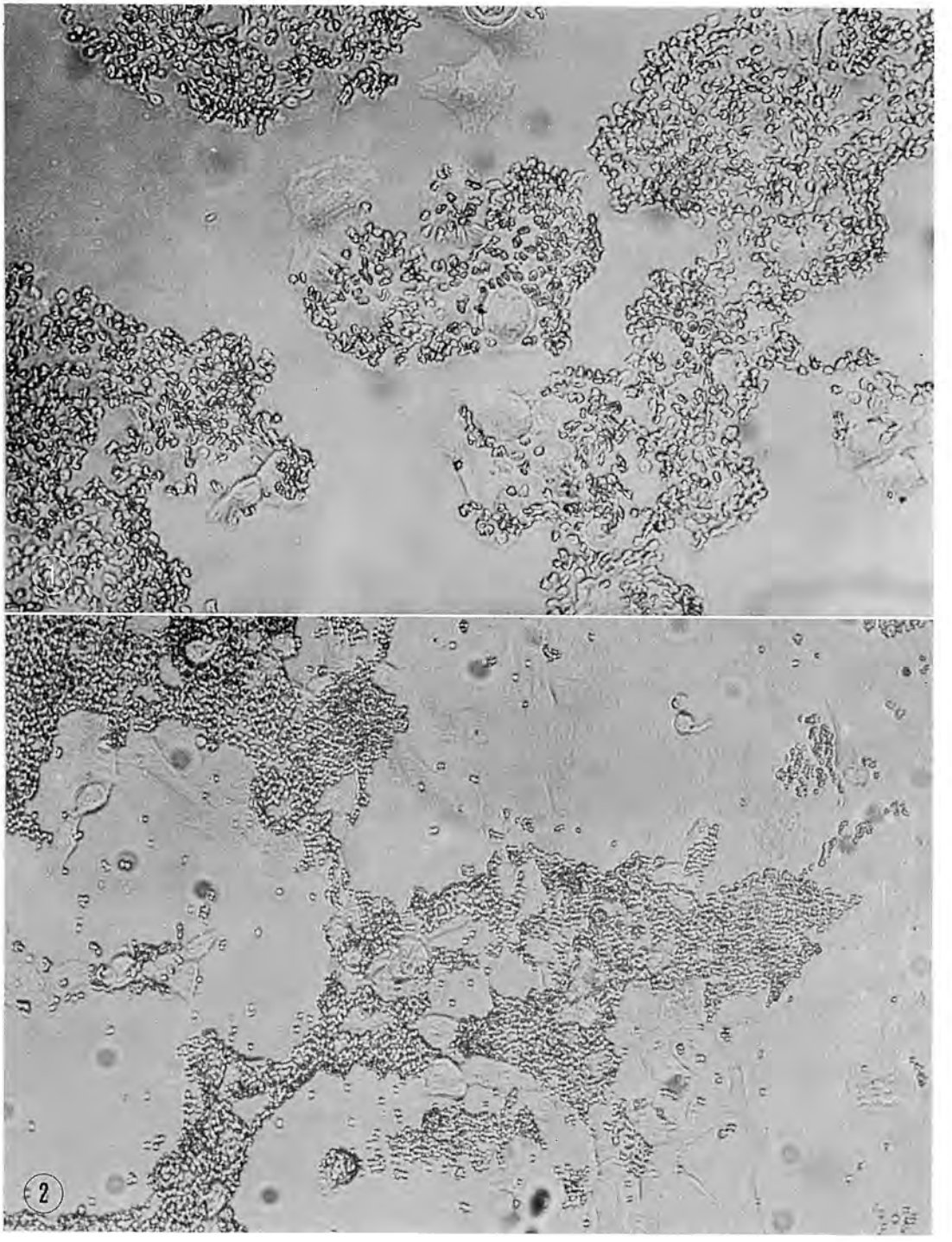
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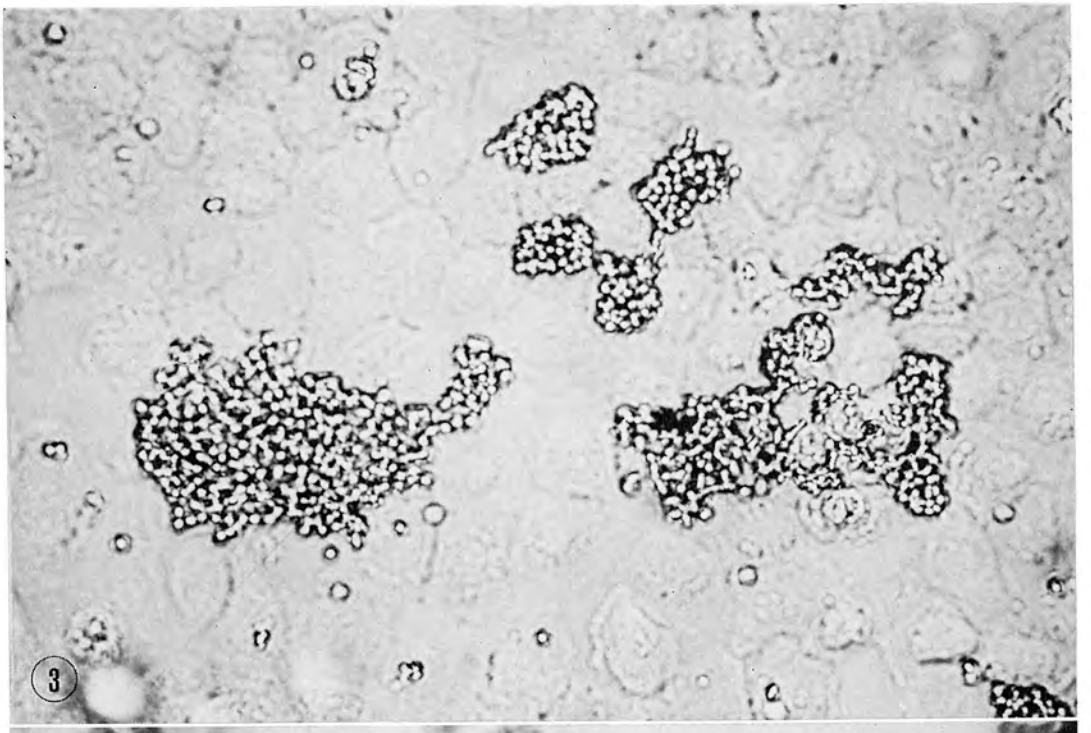
FIGURE 3 Positive immune hemadsorption on Shope fibroma virus-infected FL cells.

FIGURE 4 Cowpox viurs-infected FL cells showing both "A" and " B " type inclusion formation and adsorbed erythrocytes (Giemsa).



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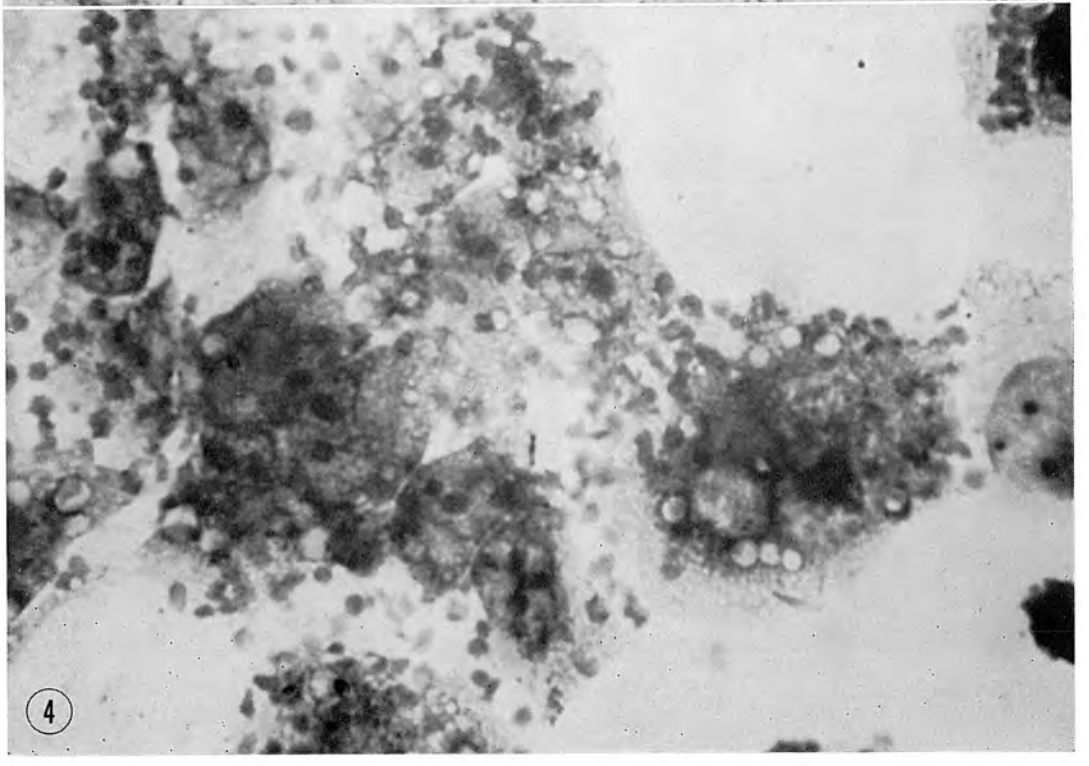

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TABLE 1 *Immune hemadsorption with Shope fibroma virus-infected cell cultures treated with various concentrations of anti-Shope fibroma virus rabbit serum*

	Indicator cells	Dilution of rabbit serum added to tubes							
		Anti-Shope fibroma virus serum						Normal rabbit serum	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-1}	10^{-2}
RK_1	C I S	−	−					−	−
	S S R	−	−					−	−
	S R	−	−					−	−
RK_1-SFV	C I S	3+	3+	3+	2+	+	−	−	−
	S S R	−	−	−				−	−
	S R	−	−	−				−	−
FL	C I S	−	−					−	−
	S S R	−	−					−	−
	S R	−	−					−	−
FL-SFV	C I S	3+	3+	3+	+	+	−	−	−
	S S R	−	−	−				−	−
	S R	−	−	−				−	−

SFV : Shope fibroma virus. CIS : 0.5% Complete indicator system.
SSR : 0.5% Sensitized sheep red blood cells. SR : 0.5% Sheep red blood cells.

TABLE 2 *Immune hemadsorption with cowpox virus-infected cell cultures treated with various concentrations of anti-cowpox virus rabbit serum*

	Indicator cells	Dilution of rabbit serum added to tubes								
		Anti-cowpox virus serum							Normal rabbit serum	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-1}	10^{-2}
FL-CPV	C I S	+	2+	2+	3+	2+	+	−	−	−
	S S R	−	−	−	−				−	−
	S R	−	−	−	−				−	−
FL	C I S	−	−	−	−				−	−
	S S R	−	−	−	−				−	−
	S R	−	−	−	−				−	−
RK_1	C I S	−	−	−	−				−	−
	S S R	−	−	−	−				−	−
	S R	−	−	−	−				−	−

CPV : Cowpox virus. CIS : 0.5% Complete indicator system.
SSR : 0..5% Sensitized sheep red blood cells. SR : 0.5% Sheep red blood cells.

TABLE 3 *Immune hemadsorption with Shope fibroma virus-infected cell cultures treated with various concentrations of anti-FL cell rabbit serum*

	Indicator cells	Dilution of rabbit serum added to tubes								
		Anti-FL cell serum							Normal rabbit serum	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-1}	10^{-2}
RK_1	CIS	−	−	−					−	−
	SSR	−	−	−					−	−
	SR	−	−	−					−	−
RK_1-SFV	CIS	−	−	−					−	−
	SSR	−	−	−					−	−
	SR	−	−	−					−	−
FL	CIS	+	2+	3+	3+	2+	+	−	−	−
	SSR	−	−	−					−	−
	SR	−	−	−					−	−
FL-SFV	CIS	+	2+	3+	3+	2+	+	−	−	−
	SSR	−	−	−					−	−
	SR	−	−	−					−	−

SFV : Shope fibroma virus.
SSR : 0.5% Sensitized sheep red blood cells.
CIS : 0.5% Complete indicator system.
SR : 0.5% Sheep red blood cells.

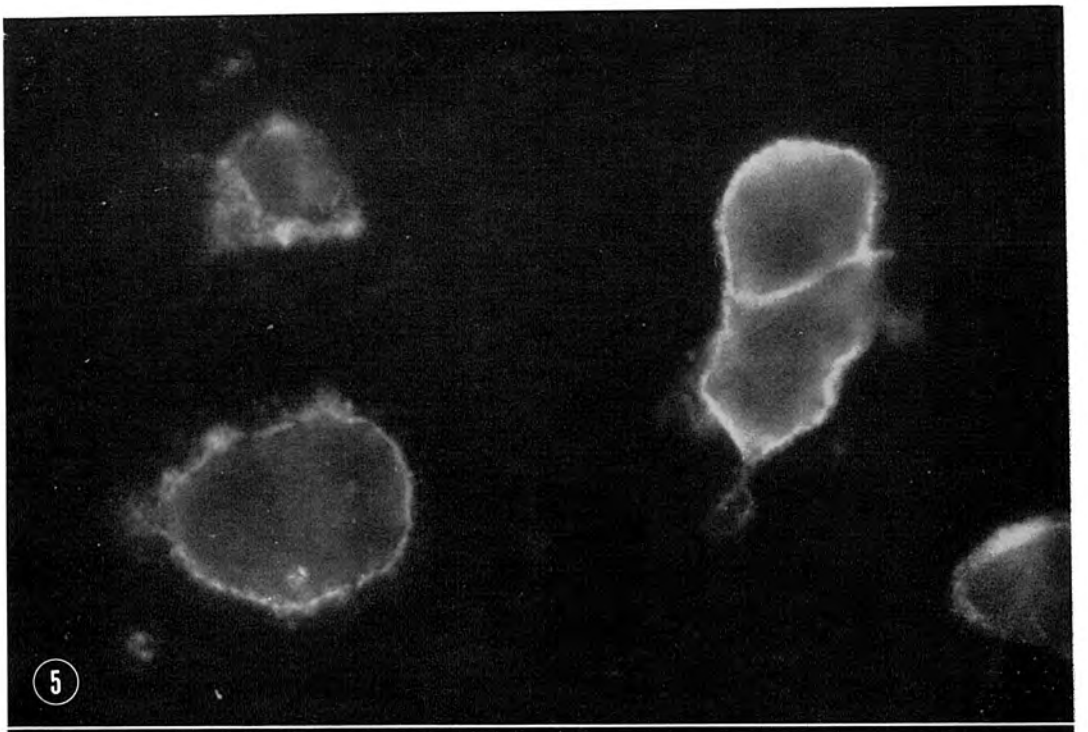
TABLE 4 *Tests for immune hemadsorption of cells infected with cowpox virus and Shope fibroma virus in the presence and abscence of cytosine arabinoside*

	Cytosine Arabinoside	DNA synthesis[a]		IHAd	FAT (nonfixed)	HAd
		"N"	"C"			
FL-CPV	−	−	3+	3+	3+	3+
	+	−	−	2+	2+	−
RK_1-SFV	−			3+	+	−
	+			2+	?	−

CPV : Cowpox virus
SFV : Shope fibroma virus
"N" : Nuclear
"C" : Cytoplasmic
IHAd : Immune hemadsorption
HAd : Hemadsorption
FAT : Fluorescent antibody technique
a DNA synthesis was studied by autoradiography of ^{3}H-thymidine.

FIGURE 5 Cowpox virus-infected FL cells were stained with fluoresceine-isothiocyanate coupled with rabbit anti-cowpox virus γ-globulin (without prefixation). Note the annular fluorescent patterns at the surface.

FIGURE 6 Cowpox virus-infected FL cells in the presence of cytosine arabinoside were stained with the same fluorescent antibody as in Fig. 5 (without prefixation). Note the similar annular fluorescent patterns at the surface.



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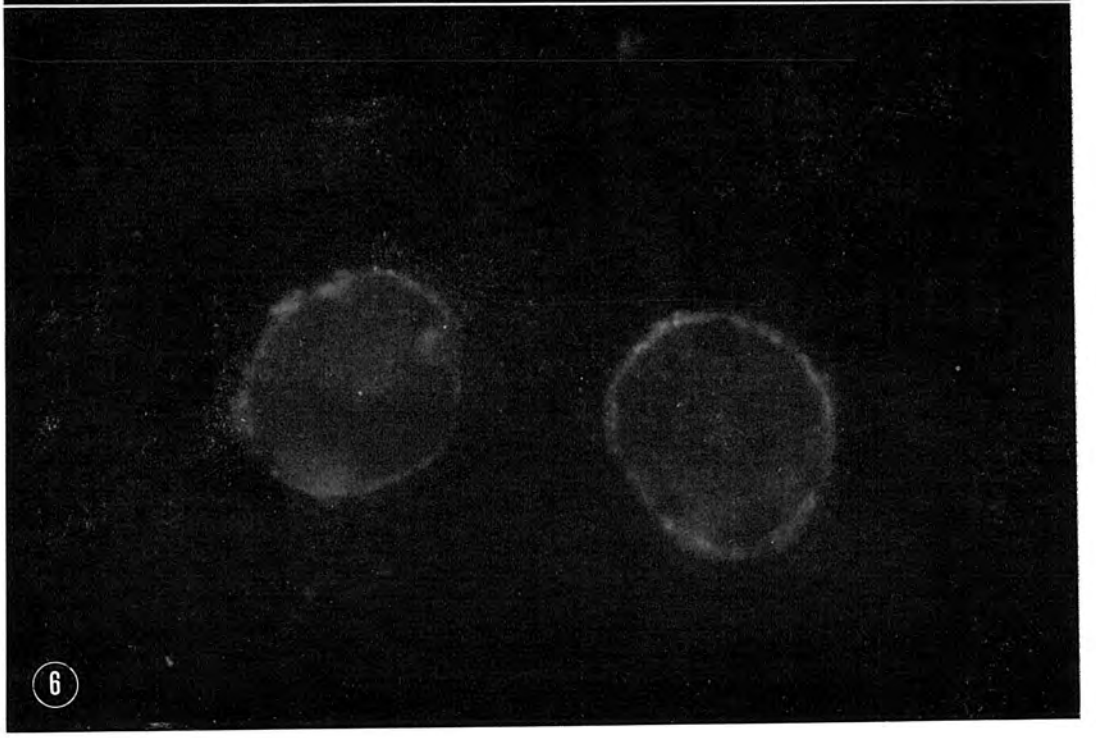

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rabbit anti-sheep erythrocyte serum and further coated with goat antiserum against rabbit γ-globulin. This antiglobulin serum against rabbit γ-globulin could form bridges between sensitized erythrocytes and infected cells. Unsensitized sheep red cells and sheep red cells sensitized with only rabbit antiserum were used as controls.

The resulting adherence of the erythrocytes to the infected cells could be detected by examination under the microscope. Observations were also made on preparations fixed with methanol, and stained with Giemsa solution after immune hemadsorption. Positive results with Shope fibroma virus-RK_1 cell, or -FL cell systems and a cowpox virus-FL cell system were obtained as shown in Table 1 and 2 respectively (Figs. 1, 2, 3, and 4). Throughout the experiments, no hemadsorption was seen in controls of the infected cells, treated with unsensitized sheep red cells or sheep red cells sensitized with only rabbit antibody. With the cowpox virus-FL cell system, a strong reaction was observed over a range of optimal concentrations of antiviral antiserum. The reasons why infected cells treated with a high concentration of antiserum gave a weak reaction remain unexplained. In both systems most of the cells showing positive immune hemadsorption simultaneously beared the " B" type inclusions of poxvirus (Fig. 4).

Rabbit anti-FL cell serum was prepared to demonstrate the effect of antibody against cellular surface antigen. Infected cells were treated with rabbit anti-FL cell serum, instead of antiviral antiserum and then the complete indicator systems were added. As shown in Table 3, FL cell antigen was demonstrated both on uninfected cells and on virus-infected cells. Using rabbit anti-cowpox virus serum, cross reactions were attempted on cells infected with two strains of vaccinia virus (Biken and Ecuador-Moscow 63), several strains of cowpox virus (50 " A "v^-, 51 "A "v^-, 58 " A "v^+ and Amsterdam " A "v^-), ectromelia virus (Hampstead " A "v^+), variola virus (Harvey) and Shope fibroma virus (OA). All the reactions were positive, although the reaction with cells infected with Shope fibroma virus was weak.

The effect of cytosine arabinoside, which inhibits viral DNA synthesis of poxvirus (ODA and JOKLIK, 1967) on cells infected with viruses was studied. As shown in Table 4, both nuclear and viral DNA synthesis in FL cells infected with cowpox virus were completely inhibited by the presence of cytosine arabinoside at a concentration of 10 μg per ml. It should, however, be noted that viral antigen, measured by the immune hemadsorption method, was detectable even in the absence of viral DNA synthesis. In the presence of cytosine arabinoside the hemadsorption of cowpox virus-infected cells with chick erythrocytes, which is mediated by viral hemagglutinin and clearly demonstrable in the absence of this compound, did not occur.

To compare results by the immune hemadsorption technique with those by the fluorescent antibody technique, fluoresceine-iso-thiocyanate coupled with anti-cowpox virus rabbit γ-globulin and with anti-Shope fibroma virus rabbit γ-globulin were prepared. Virus infected cells were washed with phosphate buffered saline. Then they were immediately exposed to homologous antiviral γ-globulin conjugated with fluorescent dye at 37°C for 1 hr. The cells were observed under a UV microscope following routine procedures for the fluorescent antibody technique. As shown in Figs. 5 and 6, annular fluorescence along the margin of the cells was clearly demonstrated. These results were in sharp contrast to the observed fluorescent areas corresponding to viral inclusions in the infected cells after fixation with acetone (KATO *et al.*, 1959, 1963).

Using rabbit anti-cowpox virus γ-globulin conjugated with fluoresceine-iso-thiocyanate, cross reactions were attempted on unfixed cells infected with two strains of vaccinia virus (Biken and Ecuador-Moscow 63), several strains of cowpox virus (50 " A "v^-, 51 " A "v^-, 58 " A "v^+ and Amsterdam " A " v^-), ectromelia virus (Hampstead " A "v^+) and variola virus (Harvey). All the reactions were positive

again. However the cross reaction on the cells infected with Shope fibroma virus remains unsolved.

Unfixed cells infected with cowpox virus in the presence of cytosine arabinoside at a concentration of 10 μg/ml also showed annular fluorescence in the fluorescent antibody technique using homologous anti-viral γ-globulin.

The relationship between the two surface antigens induced by viral infection and demonstrated by immune hemadsorption and by the fluorescent antibody technique is unknown.

ACKNOWLEDGMENTS

The authors are greatly indebted to Dr. J. YASUDA (Osaka University) for his valuable advice and help in performing the immune hemadsorption test. Goat anti-rabbit γ-globulin antiserum was kindly provided by Dr. K. KANO (State University of New York, Buffalo).

ADDENDUM

At the second symposium of the society of Japanese virologists (1968), Drs. Y. UEDA and I. TAGAYA added comments on their recent data about the detection of viral antigens present on the cell surface of vaccinia virus-infected cells by the fluorescent antibody technique. Although the details of their experiments are not known, their findings seem to agree essentially with ours.

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