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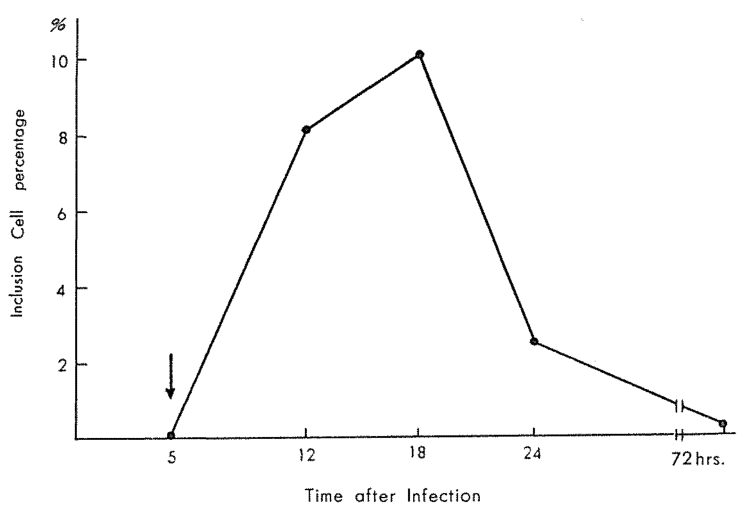
Letter to the Editor

A study on the first cycle of inclusions formed in ectromelia virus in ascites tumor cells in the abdominal cavity of rats

Studies on the one step growth cycle of animal viruses have been made by preventing secondary infection, *e. g.* by using RDE[1] or inactivated virus[2] during infection by influenza virus. The tissue culture technique permitted to increase the multiplicity of the viral infection. So many studies have been reported on the one step growth cycle of animal viruses.[3] However, there were few reports on morphological changes in the cells in the one step cycle. Poliomyelitis has been one of the most commonly studied viruses.[4,5,6] The significance of the inclusion bodies in pox group viruses is now clear. We reported Feulgen positive inclusion besides the so-called Marchal bodies and designated these as "B" type inclusion bodies.[7] The Marchal bodies were called an "A" type incluison bodies. Later, "B" type inclusions were found to be common in all pox virus infections and were the site of virus antigenicity as recognized by the fluorescein-isothiocyanate coupled antibody technique.[8,9,10,11,12] Thus pox group inclusions especially "B" type inclusion bodies can be regarded as indicators of virus infection. As might be expected, the Yoshida sarcoma cells of rats are not susceptible to the ectromelia virus of mouse introduced into the abdominal cavity of the rat. However, in the abdominal cavity of mice, the tumor cells are susceptible to the virus, forming both "A" and "B" inclusion bodies.[7] This suggested that there is a factor which inhibits some step of virus growth in the rat body.

This letter describes a study of the mechanism of the inhibitory activity of this factor in the abdominal cavity of the rat. The one step growth of the virus

Fig. 1 Inclusion Curve of Ect.-Yoshida Sarcoma System in Rat



was studied on the basis of inclusion formation under these specific conditions.

Mouse passage ectromelia virus (Hampstead strain) which had a LD50 titer of about 10^7 was used. Yoshida sarcoma cells were kindly given by Dr. Kajiwara (Takeda Pharmaceutical Co.)

The behaviour of the virus in the rat is unlike that in the mice. To analyse this differance, the following experiment was performed. Yoshida cells were mixed with virus. The mixture was introduced into the abdominal cavity of a mouse for 5 hours. Five hours was not sufficient to produce any inclusion bodies, but was sufficient to let the virus adsorb and invade the cells. Then the tumor cells were withdrawn and transferred to the abdominal cavity of a rat. At various intervals the ascites was removed by pipet. Smears of the ascites were stained with Giemsa solution and the inclusion cells were counted. As shown in Fig. 1, the inclusion cell percentage reached a maximum after 18 hours and there-after decreased rapidly. The development of the two kinds of inclusions was the same as in the Ehrlich ascites tumor cells of mice.[13] Thus, under suitable conditions, once the virus had entered the Yoshida sarcoma ascites cells viral growth proceeded in the rat. The results suggest that the apparent insusceptibility of Yoshida sarcoma cell to the virus in the abdominal cavity of the rat was not due to the nature of the cells themselves but to a factor in the abdominal cavity of the rat. Next the Yoshida tumor cells were replaced by Ehrlich ascites tumor cells, which have been extensively studied in our laboratory with regard to the virus multiplication. Ehrlich cells can survive in the abdominal cavity of the rat for more than a week. The cells were in contact with the virus in the abodminal cavity of the mouse for about 5 hours. They were then withdrawn and washed with saline. Half the cell suspension was transferred to the abdominal cavity of two rats, and half to the abdominal cavity of two mice. The two types of inclusions in the Ehrlich cells were formed in both the rats and mice. (Fig. 6). Three types of the development

Fig. 2 Inclusion Curve of Ect.-Erlich Tumor System (I)

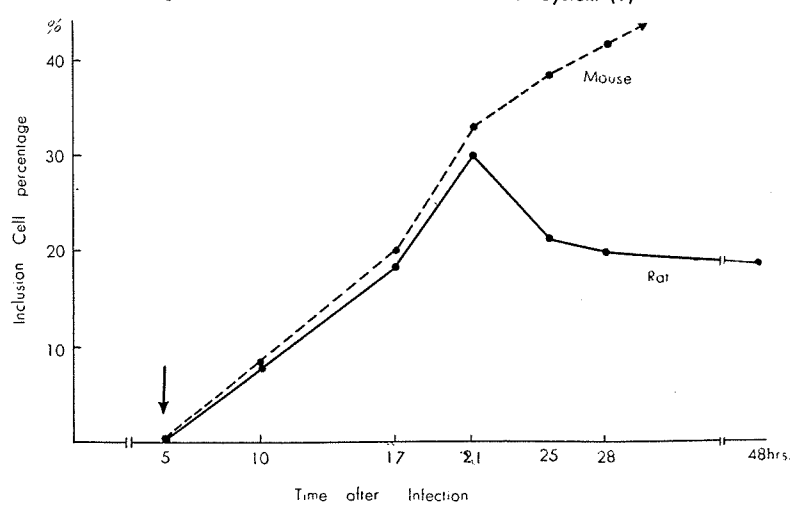
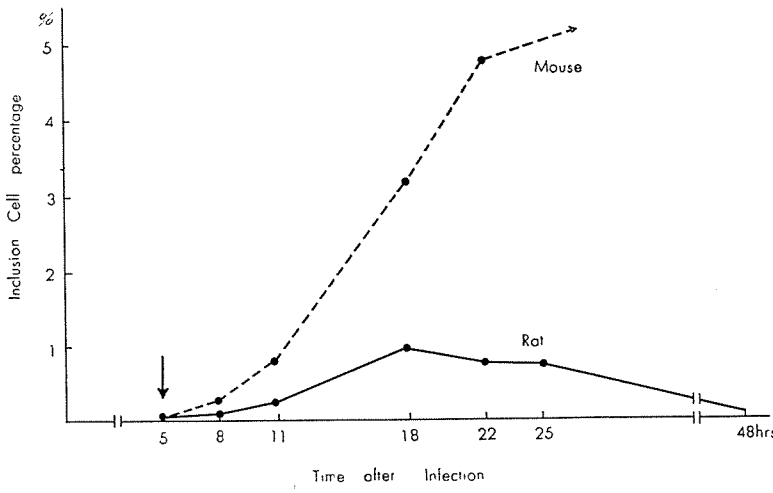


Fig. 3 Inclusion Curve of Ect.—Ehrlich Tumor System (II)



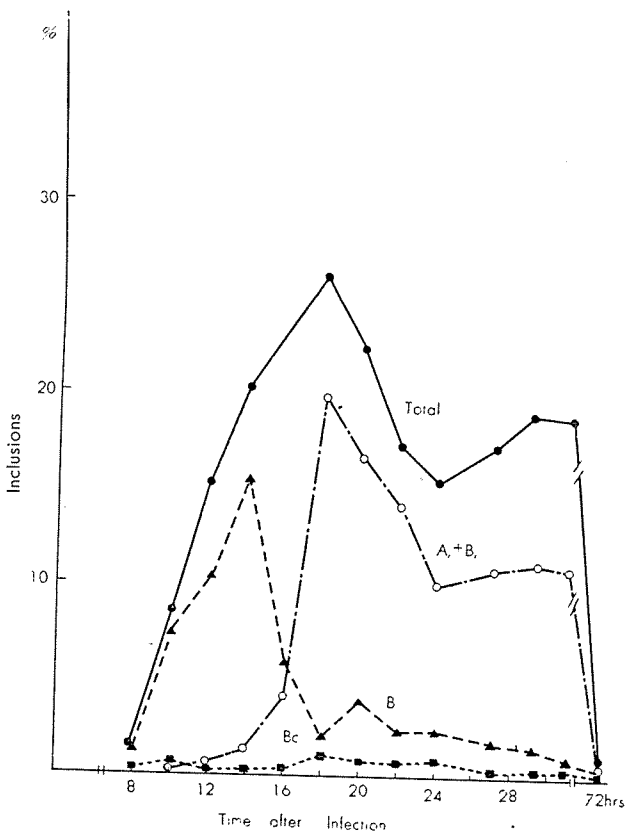


Fig. 4. Analysis of inclusion cells in rat.

Bc: Percentage of cells with small compact "B" type inclusion bodies at an early stage of the development.

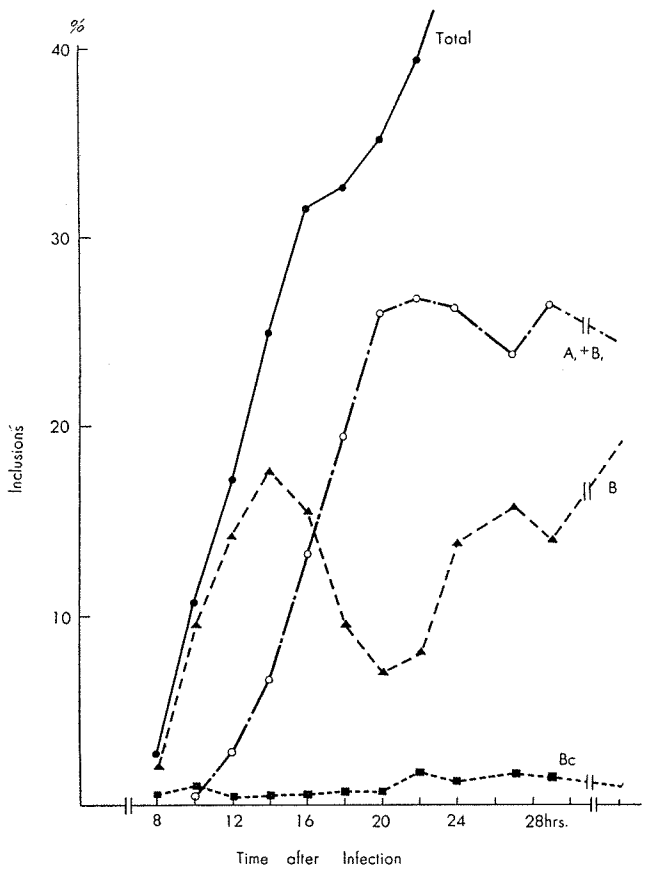
B: Percentage of cells with only "B" type inclusions.

A+B: Percentage of cells with both "A" type and the "B" type inclusions in later stages of the development.

Total: Percentage of cells with inclusion of either kind.

of inclusion formation were seen in the rats. 1) As shown in Fig. 2 the inclusion cell curve increased to 30 per cent about 20 hours after virus inoculation. This was similar to their formation in the abdominal cavity of mice. After 20 hours, inclusions gradually decreased to almost 0 per cent by about 72 hours. At this time, there were still many unchanged Ehrlich cells. This type of phenomenon was seen in about a quarter of the rats. 2) As shown in Fig. 3 although the inclusions increased steadily for 18 hours, there were considerably less than in mice. This was seen in about half the rats. 3) In the third type, oncolysis of the Ehrlich ascites tumor cells occurred soon after intraperitoneal inoculation into the rat. This occurred in a quarter of the rats. Thus the inhibition by the factor in the peritoneal fluid varied from rat to rat. The maximum inclusion cell percentage

Fig. 5. Analysis of inclusion cells in mouse.
Bc: Percentage of cells small compact "B" type inclusion bodies at an early stage of development.
B: Percentage of cells with only "B" type inclusions.
A+B: Percentage of cells with inclusion of either kind.



was always obtained about 18 hours after virus inoculation. Analysis of the inclusion bodies in type is shown in Fig. 4. The analysis was made by the method of Hagiwara *et al.* 13) A striking analogy was observed between the curves in the rat and in the mouse for 18 hours. (Fig. 5) The main difference in the curves was after 18 hours. It seems to be due to a second step cycle in inclusion formation of ectromelia virus in the mouse. There was a small increase in "Bc" type inclusions by 18 hours. It is uncertain whether inhibition by the factor was so weak that a second infection occurred or whether these was cell to cell contact infection.

In conclusion, it is most probable that there is some factor in the body fluid of the rat which prevents virus particles from entering the cells. A morphological one step growth curve based on inclusion formation of ectromelia virus was seen in the rat abdominal cavity. Inclusions of ectromelia virus can provide a useful tool for the study of the mechanism of viral growth.

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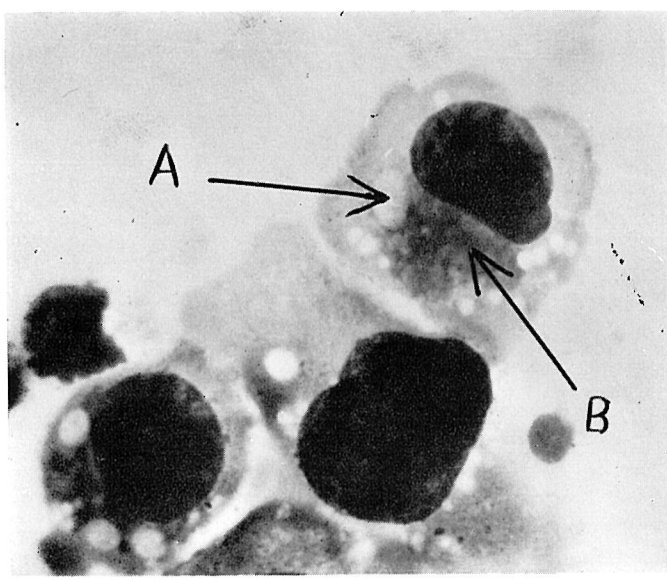

Fig. 6 "A" and "B" type inclusions in Ehrlich ascites tumor cells in the abdominal cavity of a rat. "A" bodies are pale blue, the "B" bodies reddish purple by Giemsa staining.