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Letter to the Editors

A Study on the Inhibitory Activity of 5, 6-Dichloro-1-β-D-ribofuranosyl Benzimidazole (DRB) and Proflavine on the One Step Growth Cycle of the Mousepox Virus (Ectromelia Virus) in L Cells.

There has been no biochemical analysis of nucleic acids in mousepox virus (ectromelia). However, a DNA positive inclusion designated a "B" type inclusion was found besides the so-called Marchal body ("A" type inclusion)[1]. The Feulgen positive "B" type inclusion was found common to all pox virus infections examined[2]. The "B" type inclusion is not only Feulgen positive but also a site of virus antigenicity as proved by the fluorescein isothiocyanate coupled antibody technique.[3,4] This may suggest that ectromelia virus is a DNA containing virus like other pox group viruses.

Benzimidazole derivative is known to inhibit RNA synthesis[15] and to inhibit the multiplication of influenza,[5] vaccinia,[6] and poliomyelitis virus[7]. It is reported that proflavine may prevent virus components from assembling during virus synthesis.[8, 9, 10]

Fig. 1 Effect of dose of DRB on the growth of Earle's L cells

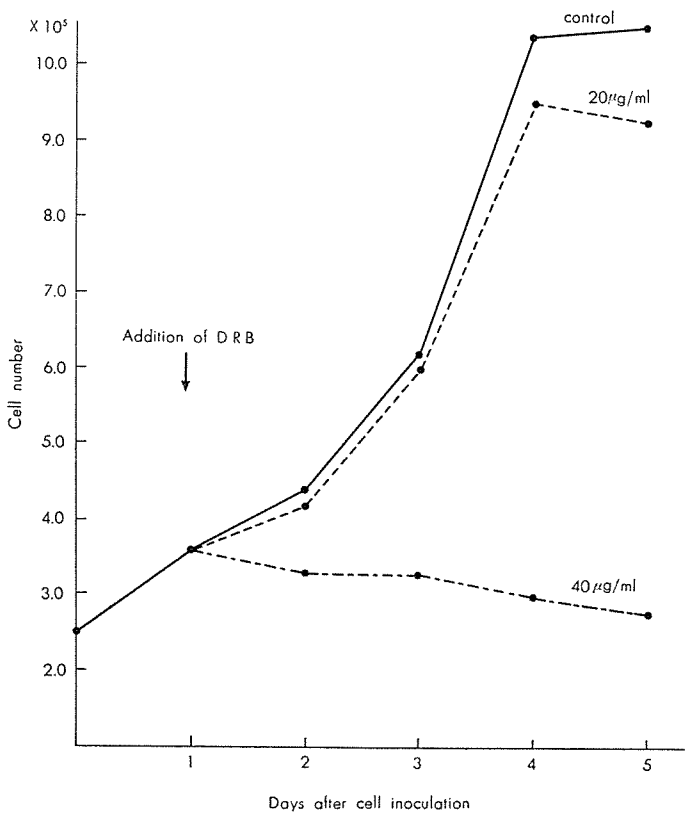
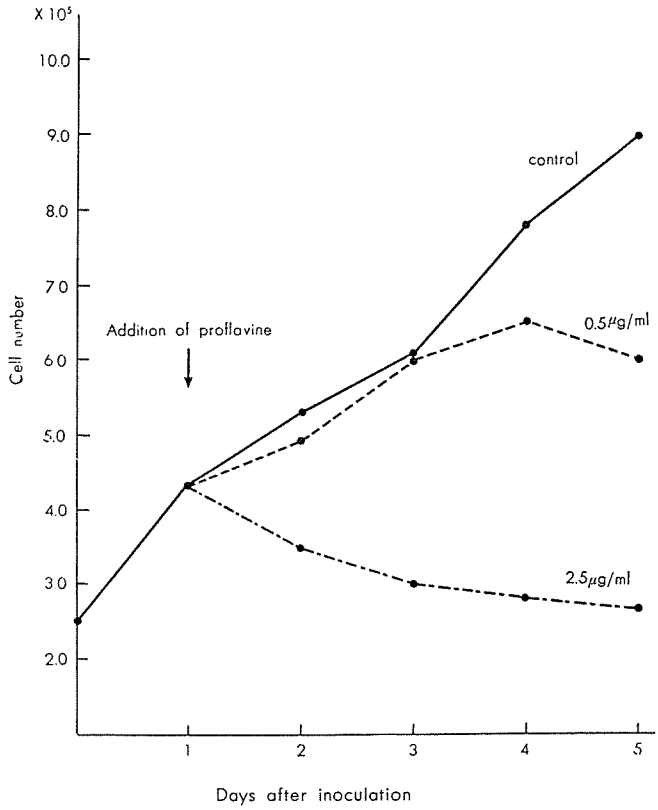


Fig. 2 Effect of dose of Proflavine on the growth of Earle's Lcells



This letter describes an analysis and comparison of the inhibitory activity of these two compounds on the "one step growth cycle" of L cells and ectromelia virus. This system has been extensively studied in our laboratory.[4, 11, 12] To analyze the action, the infectivity (plaque counting method)[12], complement fixing antigen, and inclusion cell percentage as well as the morphological, histochemical and histo-immunological characteristics of the inclusions were examined. The benzimidazole derivative, 5, 6-Dichloro-1-β-D-ribofuranosyl (DRB) was kindly provided by Dr. Karl Folkers of Merck and Company, Inc. through the courtesy of Dr. Yasuyuki Takagi of Osaka University. Proflavine was kindly given by Dr. Windsor Cutting (Stanford University). The relation between the dose of these compounds and the effect upon cell growth and morphology was shown in Fig. 1 and 2. About 2.5×10^5 cells were cultured in a series of test tubes. The culture medium consisted of 5 per cent ox serum and 95 per cent YLH (Hanks' balanced salt solution containing 0.1 per cent yeast extract and a 0.5 per cent lactalbumin hydrolysate). To obtain a one step growth curve, a virus infected mouse liver emulsion having 10^8 PFU/ml (plaque forming unit) which might infect cells at high multplicity was used. A half millilitre of viral material was

inoculated into the test tubes after two days culture.

The cells were then incubated at 37°C for two hours to allow adsorption. They were then washed three times with Hanks' solution to remove residual viruses. Samples were taken 16 hours after virus inoculation for assay. 16 hours was chosen because it was about the time when the "B" type inclusion cell percentage usually reached a maximum of more than 90 per cent.

To analyze the mechanism of the inhibition, the compound was added at various periods. In case I, the compound was added 24 hours before virus inoculation and the cells were exposed to the compound throughout the experiment. In case II, the cells were exposed to the compound only before virus inoculation to see if an influence of the compound on the condition of the host cells affected virus multiplication. In case III, cells were exposed to the compound only during virus adsorption. Case III probably shows the direct action of the compound on the virus particles and on their adsorption and invasion. In case IV, the cells were exposed to the compound from 4 to 16 hours after virus inoculation to see the effect of the compound only during the time when the viral components were being synthesized in the cell. In case V, cells were exposed to the compound from 6 hours after virus inoculation. Results were shown in Table I and II.

Table 1

Comparison of inclusion cell percentage, infectivity and complement fixing antigen in the experiments with DRB

Dose μg/ml	Case	Inclusion		Infectivitity		CFA**
		Percent	n/n_o	PFU */ml	n/n_o	
0	Control	91	1.000	1.7×10^5	1.000	64
20	I	36	0.395	1.4×10^4	0.083	32
	II	59	0.648	1.7×10^4	0.100	32
	III	91	1.000	1.7×10^5	1.000	64
	IV	89	0.978	1.6×10^5	0.940	64

Note n_o : Control value
n : Individual value
*PFU : Plaque forming unit
**CFA : Complement fixing antigen titer

There is an interesting contrast between the two compounds.

Evidently both inhibit virus multiplication. Pretreatment of the host cell with DRB suppressed all components of the multiplication of the virus; that is complement fixing antigen, inclusion body formation and plaque forming unit, as shown in the case II in Table I. However, there was little effect when the compound was added after virus inoculation. The increased turnover of RNA at the beginning of virus synthesis has been reported in bacteriophage and E. coli, and in vaccinia virus and cultured HeLa cells.[13,14] Our results suggest that DRB

Table 2

Comparison of inclusion cell percentage, infectivity and complement fixing antigen in the experiments with Proflavine

Dose μg/ml	Case	Inclusion		Infectivity		CFA**
		Percent	n/n_o	PFU*/ml	n/n_o	
0	Control	91	1.000	1.5×10^5	1.000	64
0.5	I	79	0.868	2.4×10^3	0.016	64
	II	83	0.912	4.2×10^4	0.280	64
	III	91	1.000	1.5×10^5	0.000	64
	IV	79	0.868	1.5×10^4	0.100	64
2.5	I	36	0.395	1.0×10^3	0.006	32
	II	80	0.879	2.0×10^4	0.133	64
	III	90	0.989	1.5×10^5	1.000	64
	IV	65	0.714	2.0×10^3	0.013	64
	V	70	0.769	4.0×10^3	0.026	64

Note n_o : Control value
n : Individual value
*PFU : Plaque forming unit
**CFA : Complement fixing antigen titer

probably caused some disturbance of RNA metabolism in the host cells prior to the synthesis of virus DNA. The behaviour of proflavine was rather different from that of DRB. Although it strongly suppressed infectivity of the virus, the inclusion percentage was still high and the complement fixing titer was as high as that of the control, as shown in Table 2. In proflavine treated cells, the "A" type inclusion did not appear by 16 hours and "B" type inclusions were smaller than in the control especially when more than 2.5 γ/ml of compound was added. However, the histochemical and histo-immunological characteristics of the "B" type inclusions were the same as those of the control. Thus all proflavine treated "B" type inclusions gave a Feulgen positive reaction and positive fluorescein antibody reaction at the site of the inclusion. The results seem to support the idea of De Mars[8] that proflavine may interfere either with a hypothetical "assembly mechanism" or with the synthesis of some as yet unrecognized virus constituent. Inclusion provided very useful tools for virus assay in this sort of experiment.

Details and descussion will be published in Biken's Journal.

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