ENHANCED THYMIDINE PHOSPHORYLATING ACTIVITY OF MOUSE FIBROBLASTS (STRAIN LM) FOLLOWING VACCINIA INFECTION*

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Shortly after infection of E. coli by phage, a marked enhancement of the activity of several enzymes and the acquisition by the infected cells of new enzymatic activities have been observed (Cohen, 1961; Koerner et al., 1959; Kornberg et al., 1959; Somerville et al., 1959). However, only limited data is available on the acquisition of enzymatic activity by virus infected animal cells. Rogers (1959) has described the appearance of arginase in rabbit epithelium infected by rabbit papilloma virus and Hanafusa (1961) has reported increased thymidine incorporation into DNA and enhancement of DNase activity in cell free extracts of L cells infected with vaccinia virus. In the present paper, a marked enhancement of thymidine phosphorylating activity by cell free extracts of vaccinia infected mouse fibroblasts (Strain LM) is described.

Enzyme extracts prepared from 2 day old suspension cultures of LM fibroblasts phosphorylated thymidine-H^3 at a relatively rapid rate; but extracts from 4 day old monolayer cultures of LM cells, primary rabbit kidney cells, and the tenth passage of rabbit kidney cells were considerably less active (Table 1). Extracts from 10 day old monolayer cultures did not manifest detectable enzymatic activity. This decline in thymidine phosphorylating activity with age of the culture is coincident with decreases

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in the rate of cell growth and incorporation of thymidine-H^3 into cell DNA (Kit and Dubbs, 1962).

Five hours after inoculation of vaccinia virus, enzymatic activity of suspension cultures or monolayer cultures of LM cells was markedly enhanced (Table 1).

Table 1

EFFECT OF VACCINIA (IHD) ON THYMIDINE PHOSPHORYLATING ACTIVITY OF TISSUE CULTURE CELLS AT 5 HOURS POST INOCULATION

		THYMIDINE PHOSPHORYLATED c/m/γ DNA per 10 minutes at 38° of cells exposed to:		
CELLS	DAYS AFTER SUBCULTURING	No Virus	Heated Virus*	Active Virus
LM (Suspension Cultures)	2	264	282	1330
	2	321+	353+	1630+
	2		406++	1400++
LM (Monolayer Cultures)	4	55	110++	282++
	10	0	0++	406++
Rabbit Kidney (Primary) (Monolayer Cultures)	5	67		
	10	0		
Rabbit Kidney (10th passage) (Monolayer Cultures)	4	58		
	10	0		

Twenty to 50 x 10^6 cells were frozen and thawed in one volume of 0.15M KCl - Tris buffer (pH 8) and then homogenized. The homogenate was centrifuged at 35,000 g for 1 hour and aliquots of the supernatant fraction assayed for enzymatic activity by the method of Bollum and Potter (1959). Activity was measured at three enzyme concentrations and "zero time" and "no enzyme" blanks were included with each assay. Thymidine-H^3 per flask: 1.3 x 10^5 c/m. Thymidine was separated from phosphorylated derivatives by paper chromatography using butanol-NH_3-H_2O (86:4:10) as the solvent.

* Vaccinia heated for 30 minutes at 65°.

\+ One volume of 0.05% sodium deoxycholate was added to the cells and the mixture incubated at 38° for 2 minutes. The cells were then frozen, thawed, and one volume KCl-Tris buffer, pH 8 was added. The cells were then homogenized.

++ Cells were suspended in 2 volumes of KCl-Tris buffer, pH 8, and sonicated for 8 minutes with a 9KC Raytheon ultrasonic disintegrator. Cell free extracts were then centrifuged for one hour at 35,000 g. Vaccinia which had been grown in LM cells was partially purified by centrifugation at 40,000 rpm in the Model L ultracentrifuge. The viral pellet was resuspended in the original volume of fresh growth medium. In all experiments cells were infected at a multiplicity of 5 infectious doses per cell.

A number of different methods for preparing active enzymatic extracts were investigated: 1) freezing and thawing cells followed by homogenization; 2) exposure of cells to hypotonic shock followed by freezing and thawing, and homogenization; and, 3) ultrasonic disintegration of cells. Irrespective of the method used to prepare the extracts, greatly increased enzymatic activity was observed in extracts from vaccinia infected cells. Significantly, thymidine phosphorylating activity was observed in extracts from vaccinia infected 10 day old monolayer cultures, but not from extracts of uninfected 10 day old LM cells or LM cells exposed to heat inactivated virus (Table 1).

Enzymatic activity was not detected in the cell culture medium after five hours of virus growth nor in suspensions of the virus, itself.

The enhanced enzymatic activity could be detected as early as 3 hours after virus inoculation (PI), appeared to reach a maximum 4-6 hours PI, and to decline somewhat at 7 hours PI (Table 2). It has been shown that vaccinia DNA is actively synthesized 1-6 hours PI (Salzman, 1960).

The enzymatic activity of cell free extracts of vaccinia infected cells when mixed in a 1:1 ratio with extracts from uninfected cells or cells treated with heated virus was approximately equal to the sum of the activities of each of the respective enzymatic preparations, thus contraindicating the presence of potent enzyme inhibitors in the uninfected cell extracts (Table 2).

Whether the enhanced enzymatic activity represents the acquisition of new enzyme proteins by infected cells or only an increase in host cell enzymes will be clarified following the purification of the enzymes from uninfected and vaccinia infected cells.

Table 2

ENHANCEMENT BY VACCINIA (IHD)* OF THYMIDINE PHOSPHORYLATING ACTIVITY** OF 2 DAY OLD LM SUSPENSION CULTURES AS A FUNCTION OF TIME

HOURS PI	THYMIDINE PHOSPHORYLATED c/m/γ DNA per 10 minutes at 38° by:				
	UNINFECTED CELLS	CELLS TREATED WITH:		1:1 MIXTURES OF EXTRACTS FROM	
		Heated Virus	Active Virus	Infected Cells and Uninfected Cells	Infected Cells and Heated Virus Treated Cells
1		1440	1770		
3		1180	4600		
5	1800	1000	6080	4000	3310
7		1380	3530		

* Vaccinia partially purified by centrifugation was inoculated at a multiplicity of 5 infectious doses per cell.

** Enzyme extracts prepared from cells sonicated for 8 minutes with a 9KC Raytheon ultrasonic disintegrator. Thymidine-H^3 per flask: 1.5×10^5 c/m.

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