INDUCTION OF A NEW ENZYME IN RABBIT KIDNEY CELLS BY PSEUDORABIES VIRUS*

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An increase in the activities of the enzymes involved in the synthesis of DNA after infection of cells with various viruses has been described (Green and Pina, 1962; Magee, 1962; McAuslan and Joklik, 1962; Kit and Dubbs, 1963; Nohara and Kaplan, 1963). The present communication shows that after infection with pseudorabies (Pr) virus of rabbit kidney (RK) cells in the stationary phase of growth there is an increase in the activity of these enzymes and that the thymidine monophosphate (TMP) kinase in the infected cells differs from the enzyme performing the same function in noninfected cells.

Cells were grown in Earle's saline containing 0.5% lactalbumin hydrolyzate and 5% bovine serum and were infected as described previously (Kaplan, 1957). Five hours after inoculation the cultures were washed with buffered isotonic sucrose and were collected by scraping them into a solution of sucrose (0.25 M) containing 0.004 M KCl and 0.05 M Tris pH 7.8. The cells were sonicated and centrifuged at 18,000 g for 30 minutes and the supernatant fluid (enzyme preparation) was used in all of the *in vitro* experiments.

Table I shows that the ability of infected stationary phase cells to incorporate thymidine-H^3 *in vivo* increases by approximately one

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TABLE I

Thymidine Incorporation into DNA *in Vivo* and *in Vitro*

Phase of Cell Growth		*in vivo* cpm/mg DNA x 10^3	*in vitro* µmoles/mg protein
Stationary	Noninfected	1.8	5.0
	Infected	51.0	412.0
Logarithmic	Noninfected	27.5	37.5

(a) *In vitro*: The reaction mixture (1.5 ml) contained 10 µmoles $MgCl_2$, 50 µmoles Tris buffer pH 7.8, 10 µmoles ATP, 10 mµmoles dAMP, 10 mµmoles dGMP, 10 mµmoles dCMP, 10 mµmoles thymidine, 250 µg DNA (salmon sperm), 2.5 µc thymidine-H^3 and 1 mg cell extract protein. After 2 hours of incubation at 37°, the reaction was stopped by the addition of perchloric acid (PCA) (0.5 N). Carrier DNA (500 µg) was added to the tubes and the precipitate was washed 4 times before hydrolysis in 1 ml of 0.5 N PCA at 70° for one hour. An aliquot of 0.2 ml of the hydrolyzate was transferred to 10 ml of a toluene-alcohol solution (containing 0.4% 2,5-diphenyloxazole and 0.03% 1,4-bis-2(5-phenyloxazolyl)-benzene) and the radioactivity was measured with a Packard scintillation spectrometer. (b) *In vivo*: 0.2 µc thymidine-H^3 was added to each culture, and after one hour of incubation at 37°, the cells were harvested and incorporation into DNA was estimated as above.

hundredfold by 5 hours after infection and is comparable to that of noninfected logarithmically growing cells. However, the ability of the enzyme preparations from infected stationary phase cells and noninfected logarithmically growing cells to incorporate thymidine differs by a factor of 10. The possibility was considered that this difference may result from a difference in the thermostability of the two enzyme preparations, and the next experiment shows that this is indeed the case. Fig. 1 shows that the ability of extracts from infected cells to incorporate thymidine decreases by approximately 20% after 1 hour of preincubation at 37°. Extracts from noninfected cells lose their ability to incorporate thymidine-H^3 much more rapidly. The relative thermostability of the enzyme preparation from infected cells is not

due to the presence in this preparation of protective substances, since it is not affected by a tenfold dilution with buffered isotonic sucrose nor by dialysis against 0.05 M Tris (pH 7.8) + 0.001 M mercaptoethanol for 20 hours at 4°.

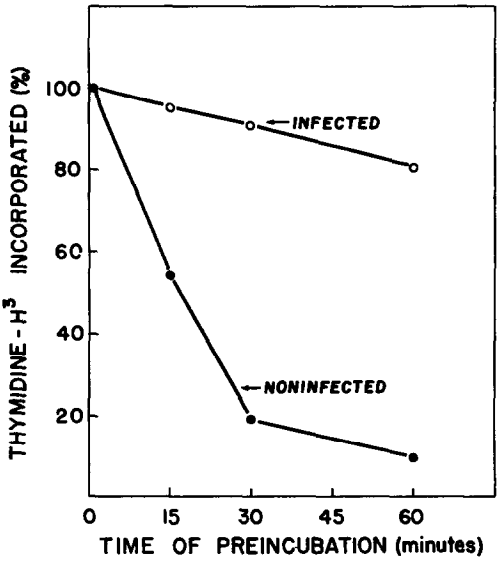

Figure 1. Ability of extracts from infected and noninfected cells to incorporate thymidine-H^3 after preincubation at 37°.

The difference in thermostability between the abilities of the enzyme preparations from infected and noninfected cells to incorporate thymidine-H^3 into DNA is due (in part, at least) to the difference between the TMP kinases from the two systems. Table II shows that preincubation for 30 minutes at 37° has little effect on the activity of thymidine kinase in either preparation; the activity of TMP kinase, however, is reduced by 90% in extracts of noninfected cells but is only slightly affected in extracts from infected cells. We have not been able to stabilize the TMP kinase from noninfected cells by the addition of TMP (Bojarski and Hiatt, 1960). Moreover, as can be expected from the incorporation studies described above, TMP kinase activity from the infected cells is not affected by dilution or by dialysis.

TABLE II

Effect of Preincubation on the Activity of Thymidine and TMP Kinases in Infected and Noninfected Cells

Extract	Treatment	Distribution of radioactivity		
		Thymidine (%)	TMP (%)	TDP-TTP (%)
Infected	None	13	58	29
	Preincubated	12	67	21
Noninfected	None	48	45	7
	Preincubated	68	32	< 0.5

Extracts were preincubated for 30 minutes at 37°. The reaction mixture (0.75 ml) contained 5 µmoles $MgCl_2$, 10 µmoles ATP, 50 µmoles Tris pH 7.8, 5.75 mµmoles thymidine, 5 µc H^3-thymidine, 1.5 mg cell extract protein. The reaction was stopped after incubation for 30 minutes at 37° by adding 0.03 ml 5 N PCA. The protein precipitate was removed by centrifugation. The supernatant was put on paper and chromatographed with an isobutyric acid-ammonia-EDTA solution (Krebs and Hems, 1953). The paper was passed through a paper strip counter and the amount of radioactivity associated with each compound was determined.

Infection of bacteria with T2 bacteriophage has been shown previously to cause the formation of deoxyribonucleotide kinases (Bello et al., 1961; Bessman and Bello, 1961), as well as a DNA polymerase (Aposhian and Kornberg, 1962), with properties quite distinct from those formed in normal bacteria. The results in this paper indicate also that the TMP kinase in Pr virus-infected HK cells is different from the enzyme performing the same function in noninfected cells.

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