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EFFECTS OF HYDROCORTISONE ON THE SYNTHESIS OF EPSTEIN-BARR VIRUS ANTIGENS IN P3HR-1 CELLS

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Summary. — Hydrocortisone was capable of enhancing the synthesis of Epstein-Barr virus antigens in producer P3HR-1 cells which had been induced with a 24-hr pulse of 5-iodo-2-deoxyuridine. The effect of hydrocortisone was time dependent, suggesting that an early step in the induction process, and not the antigen synthesis itself, was influenced by the drug.

Key words; Epstein-Barr virus; *induction*; *hydrocortisone*

Hydrocortisone (HC) increases the spontaneous Epstein-Barr virus (EBV) production in P3HR-1 cells after prolonged cultivation of the cells at decreased temperature (Magrath *et al.*, 1979) and induced spontaneous early antigen (EA) expression in RAJI cells (Joncas *et al.*, 1973). Leyritz and Joncas (1978) demonstrated similar effects of HC on several other lymphoblastoid cell lines of Burkitt lymphoma origin. Lymphoblastoid cell lines obtained from peripheral blood of healthy donors were found to be less sensitive to HC action. In the present report we describe the effects of HC added to P3HR-1 cultures treated with 5-iodo-2-deoxyuridine (IUDR).

EBV producer P3HR-1 cells cultivated as described (Vonka *et al.*, 1972) were used throughout. All experiments were run at 37 °C. Virus antigen synthesis was induced by IUDR (Koch and Light, England) at a final concentration of 20 μg/ml, which was added to the growing cultures adjusted to 4×10^5 viable cells per ml. After 24 hr (induction interval), the drug was removed and the number of cells containing virus antigens was determined immediately and on the subsequent days. The indirect immunofluorescence test was performed on acetone-fixed preperations as described by Henle and Henle (1966). Human serum from a nasopharyngeal carcinoma patient possessing antibody titres of 80 and 320 against EA and virus capsid antigen (VCA), respectively, goat anti-human IgG conjugated with fluorescein isothiocyanate (Hyland, Deerfield, U.S.A.) were used in the test. Hydrocortisone succinate (Spofa, Prague) was used at a 10^{-5} M concentration and was present either concomitantly with IUDR (i. e. during the induction period), or during the subsequent cultivation period (i. e. after IUDR removal), or in the course of both induction and subsequent cultivation periods.

HC at the concentration used did not markedly influence either the growth rate or the viability of the cells. Bottle cultures were seeded with 10 ml of a cell suspension containing 3×10^5 cells/ml. HC was added immediately after seeding. After 5 days of incubation at 37 °C, the cells were counted. Cultures

Table 1. The effect of HC on the synthesis of viral antigens in P3HR-1 cells induced with IUDR

Induction with IUDR	Treatment of the cells	Per cent of cells positive in immunofluorescence test	
		5th day	7th day
−	HC absent	2.8	2.4
+	HC absent	6.6	7.0
+	HC present during induction interval	9.8	6.9
+	HC present after IUDR removal	7.3	4.8
+	HC present continuously	13.6	8.7

grown in the absence and presence of HC contained 1.5×10^6 and 1.23×10^6 living cells per ml, the mortality having been 14.8 and 18.0%, respectively (means from 3 experiments).

As can be seen from Fig. 1, HC alone did not induce viral antigen synthesis above the level of spontaneous production. In both the presence and absence of HC the number of cells spontaneously expressing EBV antigens did not exceed 3% of the respective cell population.

The effects of HC on IUDR-treated cells are shown in Fig. 2. The percentages of cells expressing viral antigens were highest on days 5 and 7 after IUDR addition both in the presence and absence of HC. In the cultures treated with HC, the percentage of cells positive for viral antigens was more

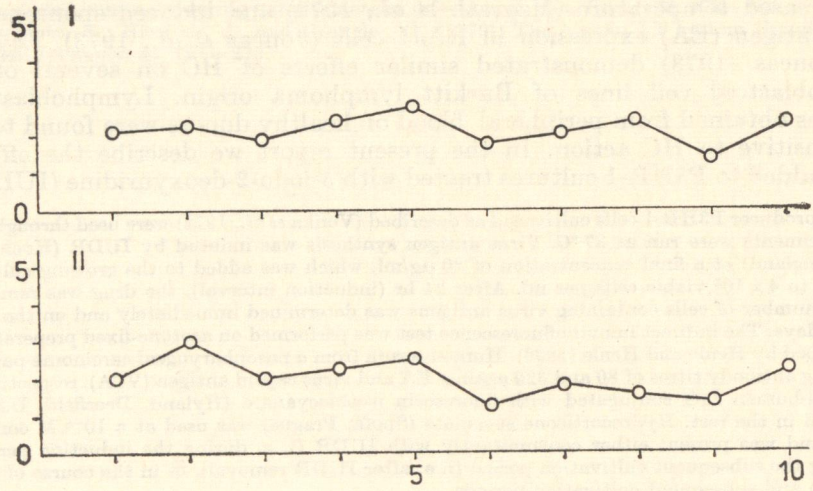

Fig. 1.
Spontaneous production of EBV antigens in P3HR-1 cells in the presence of 10^{-5} M HC (I) or its absence (II)
Abscissa: time (in days); ordinate: % of cells positive for EA + VCA

than twice higher on day 5. But subsequently the difference between the treated and untreated cultures became negligible, most probably due to lysis of cells producing viral antigens.

The results concerning the effects of the time of HC addition are summarised in Table 1. If HC was added simultaneously with IUDR and was present throughout the observation period, the percentage of cells containing

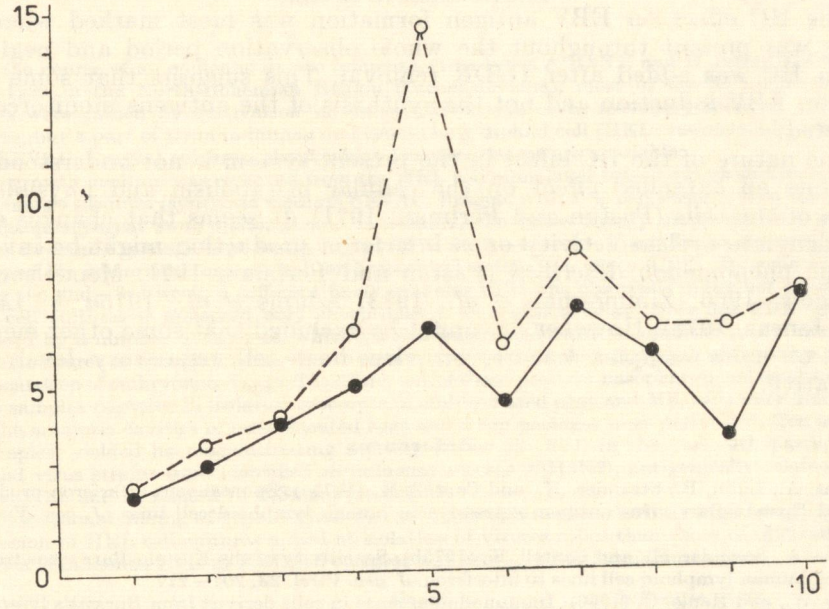

Fig. 2.

The kinetics of EBV antigen production in P3HR-1 cells after induction by IUDR HC (10^{-5} M) present (○) or absent (●)
Abscissa: time (in days); ordinate: % of cells positive for EA + VCA

viral antigens, as determined on day 5, was twice as high as in cultures treated with IUDR only. The stimulative effect of HC on the induction of viral cycle with IUDR was less pronounced when HC was added only during the induction period and no significant enhancement was demonstrated when HC was added after IUDR had been removed. Again, the rise in the percentage of positive cells was of transitory nature, in most instances it was much less marked on day 7 than on day 5.

The present data indicate that HC at the low concentration used had a stimulative effect on the synthesis of EBV antigens in P3HR-1 cells which had been induced with IUDR. HC alone did not increase expression of viral antigens in P3HR-1 cells above the level of spontaneous virus production. The induction of EBV antigen synthesis by HC in P3HR-1 cells was described by Joncas *et al.* (1973) at a higher concentration of the hormone; the con-

centration we used also proved to be ineffective in their experiments. This suggests that lower concentrations of the hormone enhance the induction of EBV antigen synthesis by IUDR while higher concentrations are necessary for direct induction of virus antigens by HC. But a significant induction of EBV antigens with low concentrations of HC has recently been achieved provided that the cells have been cultivated at 32 °C instead of 37 °C (Magrath *et al.*, 1979).

The HC effect on EBV antigen formation was most marked when the drug was present throughout the whole observation period and negligible when HC was added after IUDR removal. This suggests that some early step in EBV induction and not the synthesis of the antigens monitored was affected.

The nature of the HC effect in the present system is not understood. HC exhibits an extended effect on the cellular metabolism and physiological state of the cells (Pastan and Perlman, 1971). It seems that changes either in adenylate cyclase activity or in interferon production might be involved in the phenomenon described (Pastan and Perlman, 1971; Mendelson and Glasgow, 1966; Zimmerman *et al.*, 1973; Adams *et al.*, 1975*a*, *b*; Leyritz and Joncas, 1978). However, it cannot be excluded that some other mechanisms of more complex nature, on virus-host cell regulatory level, were operative.

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