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メタデータ	言語: eng 出版者: 公開日: 2017-10-05 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	<a href="http://hdl.handle.net/2297/29183">http://hdl.handle.net/2297/29183</a>

Key words: *HCMV DNA/dexamethasone/immediate early proteins*

## Enhanced Replication of Human Cytomegalovirus in Human Fibroblasts Treated with Dexamethasone

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(Accepted 6 June 1984)

### SUMMARY

The effect of glucocorticoid hormones on the replication of human cytomegalovirus (HCMV) was studied in human embryonic lung (HEL) cells. Treatment of cells with pharmacological concentrations of adrenal glucocorticoids such as dexamethasone enhanced HCMV replication; treatment with oestrogenic or androgenic hormones did not do so. In dexamethasone-treated HEL cells there was an approximately tenfold increase in virus yield, with the virus eclipse period shortened by 1 day compared to control cultures. Treatment of cells with the hormone also enhanced plaquing efficiency of the virus by approximately tenfold. As the synthesis of virus-specific immediate early proteins and antigens was notably enhanced together with an increase of HCMV DNA synthesis, it appeared that the early stages of the HCMV replication cycle might be under hormonal control. Moreover, the data presented suggest that the hormonal enhancement of HCMV replication involves specific receptor proteins and requires the synthesis of a specific cellular mRNA(s).

### INTRODUCTION

Herpes simplex virus (HSV) has a broad spectrum of pathogenicity and productive cytolitic activity *in vivo* and *in vitro*. In contrast, human cytomegalovirus (HCMV) infection is restricted to man and its replication *in vitro* is supported only in human diploid fibroblasts (Smith, 1959). On a molecular level, early after virus infection, HSV inhibits the synthesis of host cellular macromolecules (Ben-Porat & Kaplan, 1973). Conversely, HCMV stimulates the synthesis of cellular macromolecules and of cellular enzymes (Furukawa *et al.*, 1975*a*, 1976; St. Jeor *et al.*, 1974; Tanaka *et al.*, 1975; Hirai *et al.*, 1976; Estes & Huang, 1977). When this stimulation is inhibited chemically, by serum starvation or by u.v. light irradiation of host cells, HCMV replication is markedly reduced or eliminated (DeMarchi & Kaplan, 1977; Furukawa *et al.*, 1975*b*). These observations suggest that the replication of HCMV is dependent on stimulated host cell functions.

Adrenal glucocorticoid hormones such as dexamethasone are known to play a role in the modulation of transcription of host cell DNA. The hormones first bind to a specific receptor protein present in cytoplasm (Yamamoto & Alberts, 1976) and then these hormone-receptor complexes bind to cellular DNA (Jensen *et al.*, 1968; Gorski *et al.*, 1968). This interaction has significant effects on synthesis and translation of host cell mRNA (Higgins & Gehring, 1978). Therefore, it is of interest to study whether these hormones affect the replication of HCMV in the permissive system.

In this report we describe the effect of glucocorticoid hormones on the replication of HCMV. The results clearly show that treatment of human embryonic lung fibroblasts with pharmacological concentrations of dexamethasone significantly enhances HCMV replication.

## METHODS

*Cells and virus.* Human embryonic lung (HEL) cells, prepared from 4-month-old foetal lung by explanting minced tissue, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum. HCMV-infected HEL cells were maintained with DMEM containing 5% calf serum. The Towne strain of HCMV was used in these studies. Stocks of virus were prepared by infecting confluent monolayers of HEL cells with HCMV at a multiplicity of infection (m.o.i.) of 0.01 p.f.u./cell. Three to 5 days after cytopathic effect (c.p.e.) had developed in 100% of the cells, culture media were harvested and clarified by centrifugation (1500 g for 15 min). The supernatants were collected and stored at  $-85^{\circ}\text{C}$ . Cells and virus were found to be free of mycoplasma contamination by inoculation into PPLO broth (Difco) as described previously (Stinski, 1976).

*Treatment of HEL cells with corticosteroid hormones.* Stock solutions of hormones were prepared in 99.5% ethanol. The hormones, when present, were given 24 h before virus infection. The cells were washed three times with Hanks' balanced salt solution (HBSS), virus was inoculated and maintenance medium or agarose overlay medium (for plaque assay) containing hormones was added. Control cultures were similarly treated with DMEM containing amounts of ethanol equivalent to those in the hormone-treated cultures. All hormones used in these experiments were obtained from Sigma.

*Titration of infectious virus.* Semi-confluent monolayers of HEL cells grown in 1 oz prescription bottles were infected with HCMV. After a 90 min adsorption period, the cells were washed twice with HBSS and maintenance medium was added. At various intervals post-infection, the total amount of infectious virus was measured after the cells had been disrupted by freezing and thawing once, and by sonication (Branson sonifier) for 30 s. The infectious virus titre was determined on HEL cells using the plaque assay described by Wentworth & French (1970).

*DNA labelling and analysis.* The method used for DNA analysis was, in general, that described by Crouch & Rapp (1972). HEL cells grown in 4 oz prescription bottles were infected with HCMV at an m.o.i. of 1. After 90 min adsorption, the cells were washed with HBSS and maintenance medium was added. The cultures were labelled with [ $Me\text{-}^3\text{H}$ ]thymidine (5  $\mu\text{Ci/ml}$ ; sp. act. 101 Ci/mmol; New England Nuclear) at 24 h post-infection and incubated for an additional 24 h. The cells were rinsed three times with ice-cold TES buffer (10 mM-Tris-HCl pH 7.4, 10 mM-EDTA, 50 mM-NaCl), lysed in 1% Sarkosyl NL97 for 15 min at  $60^{\circ}\text{C}$ , and then digested by Pronase (final concentration 5 mg/ml) for 2 h at  $37^{\circ}\text{C}$ . A 0.5 ml aliquot of the sample was mixed with 9.5 ml of CsCl (initial density 1.700 g/ml), prepared in TES buffer, and centrifuged at 28000 r.p.m. for 64 h at  $18^{\circ}\text{C}$  in the 50Ti rotor. Fractions were collected from the top of the gradient by means of a density gradient fractionator (Gilson), and radioactivity incorporated into 10% trichloroacetic acid-insoluble material was counted in a Beckman scintillation counter.

*Polyacrylamide gel electrophoresis (PAGE).* HEL cells grown in 60 mm Petri dishes were infected with HCMV at an m.o.i. of 1 or 5, or mock-infected. After 1 h adsorption, the cells were washed twice with HBSS and then labelled with 30  $\mu\text{Ci}$  [ $^{35}\text{S}$ ]methionine (sp. act. 1110 Ci/mmol, Amersham) per ml for 2 h in methionine-free maintenance medium. The cultures were washed three times with ice-cold phosphate-buffered saline. Immediate early polypeptides were extracted, precleared of extraneous protein by incubation with HCMV-negative human serum and then immunoprecipitated by immediate early antigen (IEA)-positive human serum according to a method described previously (Michelson *et al.*, 1979; Blanton & Tevethia, 1981). Immunoprecipitated polypeptides were separated by electrophoresis on 10% slab gels (Laemmli, 1970). After electrophoresis, gels were fixed, soaked in En $^3$ Hance (New England Nuclear) and dried. The autoradiogram was recorded on Kodak X-Omat film. [ $^{35}\text{S}$ ]Methionine-labelled Sendai virus structural polypeptides were co-electrophoresed on each gel as molecular weight standards.

## RESULTS

*Effect of dexamethasone on plaquing efficiency and production of HCMV*

To study the effect of adrenal corticosteroids on the multiplication of HCMV, we first determined plaquing efficiency and production of HCMV in HEL cells treated with various concentrations of dexamethasone, a synthetic glucocorticoid. As shown in Table 1, plaque numbers found of HEL cells treated with  $10^{-5}$  to  $10^{-8}$  M-dexamethasone were five- to 11-fold higher than those on untreated control cultures. In addition, individual plaques appeared earlier and were larger on the hormone-treated HEL cells as compared with untreated cultures; plaques first appeared after 4 days on  $10^{-5}$  M-dexamethasone-treated HEL cells in contrast to the first appearance at 7 days post-infection on control cultures; plaque size on the hormone-treated cultures was 1.5- to 2-fold larger than that on the control when measured at 14 days. The yield of HCMV in the hormone-treated HEL cells was also enhanced 2.5- to 14.3-fold over production in untreated cultures (Table 1).

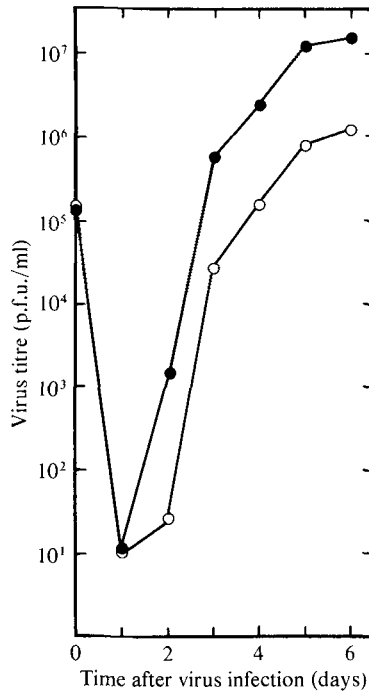


Fig. 1. Growth kinetics of HCMV in untreated or dexamethasone-treated HEL cells. The cells either untreated or pretreated for 24 h with the hormone ( $10^{-5}$  M) were infected with HCMV at an m.o.i. of 1. After 90 min adsorption the cells were washed with HBSS and maintenance medium with or without the hormone was added to the respective cells. At the indicated times after virus infection, the total amount of infectious virus was determined by plaque assay. Medium was changed at 3 days. ○, Untreated control cultures; ●, dexamethasone-treated cultures.

Table 1. *Effect of various concentrations of dexamethasone on plaquing efficiency and production of HCMV*

Dexamethasone (M)	No. of plaques per plate*	Virus yield† (p.f.u./ml $\times 10^{-4}$ )
0	6	2.2
$10^{-8}$	30	5.5
$10^{-7}$	42	12.5
$10^{-6}$	51	30.5
$10^{-5}$	66	31.5

\* Confluent monolayers of HEL cells, grown in 60 mm Petri dishes, were pretreated for 24 h with the indicated concentration of dexamethasone. The cells were infected with HCMV at an m.o.i. of approx. 10 p.f.u./plate. After 90 min adsorption the plates were overlaid with maintenance medium containing 0.4% agarose and the indicated concentration of the hormone. Seven days later a second overlay was added to the plates. The plaques were scored at 14 days; average plaque numbers in each of two samples are shown.

† HEL cells pretreated for 24 h with the indicated concentration of dexamethasone were infected with HCMV at an m.o.i. of 1. After 90 min adsorption, maintenance medium containing the indicated concentration of the hormone was added. At 72 h post-infection the total amount of infectious virus was determined.

#### *Growth kinetics of HCMV in untreated or dexamethasone-treated HEL cells*

Growth of HCMV in either untreated or the hormone-treated cultures was assessed by infecting monolayers at an m.o.i. of 1. A typical experiment is shown in Fig. 1. The synthesis of infectious HCMV was first observed at 3 days in untreated control cultures. In contrast, in the hormone-treated cultures infectious progeny virus appeared 2 days after infection and the amount of infectious virus produced during 3 to 6 days post-infection was consistently about 10-fold higher than that in the control.

Table 2. *Effect of various steroid hormones on replication of HCMV in HEL cells\**

Expt. no.	Hormone†	Virus yield‡ (p.f.u./ml × 10 <sup>-3</sup> )
1	None	3.7
	Dexamethasone	127.5
	Hydrocortisone	24.5
	Prednisolone	22.5
	Progesterone	7.0
	β-Oestradiol	6.5
	Dexamethasone + progesterone	62.5
2	None	7.5
	Hydrocortisone	51.7
	Progesterone	7.7
	Testosterone	7.0
	Hydrocortisone + progesterone	24.5

\* HEL cells either untreated or pretreated for 24 h with the indicated steroid hormone were infected with HCMV at an m.o.i. of 0.1. After 90 min adsorption, maintenance medium containing the indicated hormone was added.

† The concentration of each hormone was 10<sup>-6</sup> M.

‡ Titrated 72 h after virus infection.

#### *Effect of other steroid hormones on production of HCMV*

To determine whether this enhancing activity by dexamethasone is due to glucocorticoid activity, experiments using other steroid hormones were carried out (Table 2). Dexamethasone enhanced the production of HCMV 34.4-fold over that in untreated cultures. Two natural glucocorticoid hormones enhanced the production of HCMV by levels ranging from 6.0-fold (prednisolone) to 6.8-fold (hydrocortisone). However, oestrogenic (progesterone and β-oestradiol) and androgenic (testosterone) hormones did not enhance HCMV multiplication. Because progesterone has been shown to act as a glucocorticoid hormone antagonist by competing for cytoplasmic receptor protein (Samuels & Tomkins, 1970), we tested the effect of progesterone on the enhancement of HCMV production by dexamethasone or hydrocortisone. The enhancing activity by dexamethasone or hydrocortisone was reduced by 50% when infected cells were treated simultaneously with these hormones and progesterone (Table 2). These results indicate that the mechanism resulting in enhanced production of HCMV is specific for adrenal glucocorticoid hormones and suggest that the hormonal enhancement of HCMV production involves specific receptor proteins in target cells.

#### *Virus adsorption and cell growth*

A possible mechanism involved in the enhanced production of HCMV by dexamethasone is hormone enhancement of virus adsorption or stimulation of cell growth. It is known that HCMV replication in growing cells is faster and greater than that in resting cells (DeMarchi & Kaplan, 1977). To test this possibility, the rate of cell growth and virus adsorption were examined. No significant difference was observed in the cell growth in either untreated or hormone-treated cells. To determine the adsorption rate of HCMV, monolayers of HEL cells, either untreated or pretreated with dexamethasone, were exposed to partially purified HCMV that had been labelled with [<sup>3</sup>H]thymidine. The cultures were incubated at 37 °C. At 30 min intervals the inoculum was removed from each of two samples and residual acid-insoluble radioactivity in the supernatant was counted. Pretreatment of HEL cells with the hormone did not affect the rate of virus adsorption during the 90 min adsorption period (data not shown).

#### *Analysis of HCMV DNA synthesis in dexamethasone-treated cells*

The rate of HCMV DNA synthesis in dexamethasone-treated HEL cells was compared to that in untreated control cultures. HEL cells untreated or pretreated with dexamethasone were

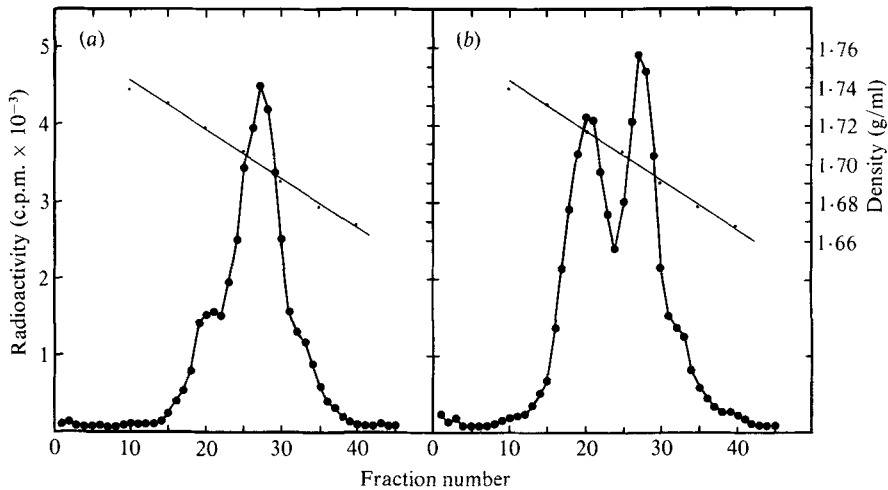


Fig. 2. Analysis of DNA isolated from untreated or dexamethasone-treated HEL cells infected with HCMV. HEL cells either untreated or pretreated for 24 h with the hormone ( $10^{-5}$  M) were infected with HCMV at an m.o.i. of 1. The cells were pulse-labelled with [ $^3$ H]thymidine from 24 to 48 h after infection in the presence or absence of the hormone. DNA was extracted and virus DNA was separated from cellular DNA by isopycnic centrifugation in CsCl as described in Methods. The amount of [ $^3$ H]-thymidine incorporated into acid-insoluble material was determined. (a) DNA extracted from untreated control cultures; (b) DNA extracted from dexamethasone-treated cultures.

Table 3. Production of HCMV in HEL cells treated with dexamethasone at various times before and after virus infection\*

Period of dexamethasone treatment (h)†	Virus yield‡ (p.f.u./ml $\times 10^{-5}$ )
Untreated	3.0
-24-0	13.2
1.5-96	28.5
24-96	18.7
48-96	7.7
72-96	5.5
-24-0, 1.5-96	37.0

\* HEL cells were treated with dexamethasone ( $10^{-5}$  M) for the indicated period before and after virus infection at an m.o.i. of 1.

† The time of virus infection is taken as 0 time.

‡ Titrated at 96 h post-infection.

infected with HCMV at an m.o.i. of 1, and pulsed with [ $^3$ H]thymidine from 24 to 48 h post-infection in the presence or absence of the hormone. Virus DNA and host cell DNA in cellular extracts were separated by equilibrium centrifugation in CsCl as described in Methods. Fig. 2 illustrates the sedimentation profiles. In the hormone-treated cultures (Fig. 2b) the peak (density 1.717 g/ml) that corresponds to the density of standard HCMV DNA (Plummer *et al.*, 1969; St. Jeor & Rapp, 1973) was about three times larger than that in control cultures (Fig. 2a). However, the peak at the density of mammalian cell DNA (Crouch & Rapp, 1972) was not significantly different between the two cases. These results indicate that the synthesis of virus DNA is enhanced in the dexamethasone-treated HEL cells.

To determine the stages in the HCMV replication cycle that are under hormonal control, HEL cell monolayers were treated with dexamethasone at various times before and after virus infection and virus yield was determined at 96 h (Table 3). The yield of virus was significantly enhanced in cultures treated with the hormone prior to virus infection (-24 to 0 h) or during the 48 h latent period of virus replication (1.5 to 96 h and 24 to 96 h). However, a little enhancement

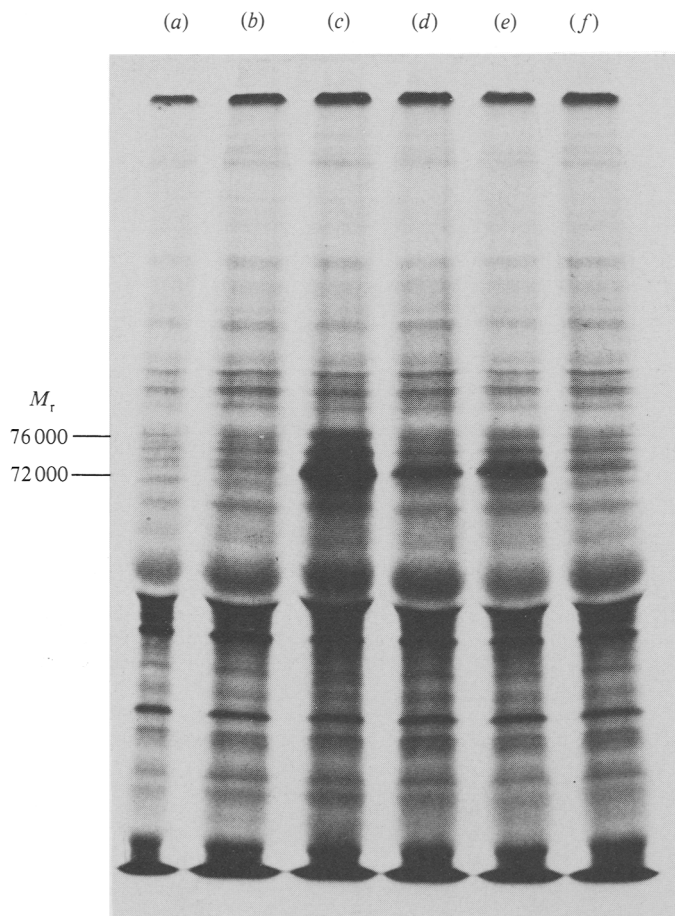


Fig. 3. Analysis of immediate early proteins synthesized in untreated or dexamethasone-treated HEL cells by SDS-PAGE. HEL cells were either untreated or pretreated for 24 h with the hormone ( $10^{-5}$  M). These cells were infected with HCMV at an m.o.i. of 1 (*e, f*) or 5 (*c, d*), or mock-infected (*a, b*). After a 1 h adsorption period the cells were washed and pulse-labelled with [ $^{35}$ S]methionine for 2 h in methionine-free maintenance medium with or without the hormone. (*b, d, f*) Untreated control cultures; (*a, c, e*) dexamethasone-treated cultures.

was observed in cultures treated with the hormone after virus synthesis began (48 to 96 h and 72 to 96 h).

*Effect of dexamethasone on the synthesis of immediate early proteins and antigens*

During the course of these experiments, we noticed that in the hormone-treated HEL cells early c.p.e., resulting from the synthesis of early proteins (Michelson-Fiske *et al.*, 1977; Stinski, 1978), appeared earlier and in more cells than in untreated control cultures. Therefore, we examined the synthesis of immediate early proteins in the hormone-treated cells by SDS-PAGE analysis. The results, illustrated in Fig. 3, show that IEA-positive human serum mainly precipitated a band with an apparent molecular weight of 72000 in all virus-infected cells (lanes *c* to *f*), but not in either untreated or dexamethasone-treated mock-infected cells (lanes *a* and *b*). The relative amount of synthesis of the protein was enhanced in the hormone-treated cultures infected with HCMV at an m.o.i. of 5 (lane *c*) or 1 (lane *e*) compared to the respective control cultures (lanes *d* and *f*). Moreover, in the hormone-treated cultures infected with virus at an

Table 4. *Effect of cordycepin on enhancing activity by dexamethasone\**

Dexamethasone (M)	Cordycepin ( $\mu\text{g/ml}$ )	Virus yield† (p.f.u./ml $\times 10^{-3}$ )
0	0	1.2
0	20	2.2
0	30	1.4
$10^{-5}$	0	8.7
$10^{-5}$	20	3.5
$10^{-5}$	30	1.8

\* HEL cells were pretreated with the indicated concentration of dexamethasone, cordycepin or both for 24 h before virus infection at an m.o.i. of 0.1.

† Titrated at 72 h post-infection.

m.o.i. of 5, another polypeptide (apparent mol. wt. 76000) was also found in addition to a 72000 polypeptide (lane *c*).

The synthesis of IEA was also stimulated in the hormone-treated cells. HEL cells infected with virus at an m.o.i. of 0.1 were fixed with methanol at 3 h and stained by standard indirect immunofluorescence techniques (Tanaka *et al.*, 1981) using the same serum as used in the immunoprecipitation. In untreated control cultures, IEA was found in 1 to 3% of the cells. In contrast, in the hormone-treated cells 8 to 10% of the cells contained IEA.

#### *Effect of cordycepin on the enhancement of HCMV production by dexamethasone*

To determine whether the enhancement of HCMV production by dexamethasone is dependent on the synthesis of a cellular mRNA(s) as a result of hormonal action, like hormonal enzyme induction (Scott *et al.*, 1972), the effect of cordycepin on the enhanced production of HCMV in the hormone-treated cultures was studied. This experiment is based on the fact that cordycepin inhibits the maturation of mRNA by blocking poly(A) synthesis (Penman *et al.*, 1970; Darnell *et al.*, 1971; Abelson & Penman, 1972). The results are shown in Table 4. When cells were pretreated with dexamethasone, the yield of HCMV was enhanced by approximately sevenfold. Pretreatment with cordycepin alone did not affect the virus yield. In the cultures pretreated simultaneously with the hormone and cordycepin at concentrations of 20 or 30  $\mu\text{g/ml}$ , there was about 60 or 80% reduction in virus yield when compared to the yield in the cells treated with dexamethasone alone.

#### DISCUSSION

The data presented in this paper indicate that treatment of cells with pharmacological concentrations of dexamethasone enhances HCMV replication in HEL cells. This enhanced replication of HCMV is demonstrated by several biological and biochemical parameters, i.e. enhanced plaquing efficiency, earlier appearance of c.p.e., shortened virus eclipse period, increased synthesis of immediate early proteins and antigens, and enhanced virus DNA synthesis.

In these studies we have found that the hormonal enhancement of HCMV replication probably involves specific receptor proteins in target cells and requires the synthesis of a cellular mRNA(s). Because androgenic and oestrogenic hormones do not significantly enhance HCMV replication, this involvement of transcription in enhancement may be specifically induced by glucocorticoid hormones. This may be because androgenic and oestrogenic hormones have different modes of action or because fibroblast cells are not targets for these two hormones.

In our system, the early stages in HCMV replication cycle seem to be under hormonal control. It is likely that the enhancing effect of the hormone is expressed through host cellular functions. Evidence that this may be the case comes from the fact that when cultures are pretreated with the hormone, which is then removed before infection, the yield of progeny virus is significantly higher than that in untreated control cultures (Tables 3 and 4). However, the possibility that dexamethasone may stimulate HCMV transcription directly can not be ruled out, because the



yield of HCMV is further enhanced when the hormone is allowed to remain in the cultures throughout the experiment (Table 3). This problem is now under investigation.

The same hormone has been found to enhance production of murine leukaemia virus (Paran *et al.*, 1973), murine mammary tumour virus (Parks & Scolnick, 1974), polyoma virus (Morhenn *et al.*, 1973) and a certain strain of HSV type 2 (Costa *et al.*, 1974). However, whether these phenomena have similar molecular mechanisms is unknown.

Under natural conditions, HCMV causes many diseases after primary or reactivation infection. Chiba *et al.* (1972) have suggested that corticosteroid hormones are one risk factor for HCMV infection in children. Another cytomegalovirus, murine cytomegalovirus, has been shown to be reactivated from latent virus infection by treatment of the animals with cortisone and anti-lymphocyte serum (Jordan *et al.*, 1977). Moreover, recently we have found that dexamethasone also enhances HCMV replication in human epithelial cells in which HCMV replication occurs most frequently *in vivo* (Tanaka *et al.*, 1984). These observations, coupled with the findings described here, suggest that glucogenic steroid hormones could play a biologically significant role in HCMV-cell interactions *in vivo*.

#### REFERENCES

- ABELSON, H. T. & PENMAN, S. (1972). Messenger RNA formation: resistance to inhibition by 3'-deoxycytidine. *Biochimica et biophysica acta* **277**, 129-133.
- BEN-PORAT, T. & KAPLAN, S. (1973). Replication - biochemical aspects. In *The Herpesviruses*, pp. 163-220. Edited by A. S. Kaplan. New York: Academic Press.
- BLANTON, R. A. & TEVETHIA, M. J. (1981). Immunoprecipitation of virus-specific immediate-early and early polypeptides from cells lytically infected with human cytomegalovirus strain AD169. *Virology* **112**, 262-273.
- CHIBA, S., IKEDA, S., CHIBA, Y., AGATSUMA, Y., OHSAKI, M. & NAKAO, T. (1972). Epidemic of subclinical infection with cytomegalovirus in an infant home. *Tohoku Journal of Experimental Medicine* **106**, 387-389.
- COSTA, J., YEE, C., TROOST, T. & RABSON, A. S. (1974). Effect of dexamethasone on herpes simplex virus type 2 infection *in vitro*. *Nature, London* **252**, 745-746.
- CROUCH, N. A. & RAPP, F. (1972). Cell-dependent differences in the production of infectious herpes simplex virus at a supraoptimal temperature. *Journal of Virology* **9**, 223-230.
- DARNELL, J. E., PHILIPSON, L., WALL, R. & ADESNIK, M. (1971). Polyadenylic acid sequences: role in conversion of nuclear RNA into messenger RNA. *Science* **174**, 507-510.
- DEMARCHI, J. M. & KAPLAN, A. S. (1977). Physiological state of human embryonic lung cells affects their response to human cytomegalovirus. *Journal of Virology* **23**, 126-132.
- ESTES, J. E. & HUANG, E. S. (1977). Stimulation of cellular thymidine kinases by human cytomegalovirus. *Journal of Virology* **24**, 13-21.
- FURUKAWA, T., TANAKA, S. & PLOTKIN, S. A. (1975a). Stimulation of macromolecular synthesis in guinea pig cells by human CMV. *Proceedings of the Society for Experimental Biology and Medicine* **148**, 211-214.
- FURUKAWA, T., TANAKA, S. & PLOTKIN, S. A. (1975b). Restricted growth of human cytomegalovirus in uv-irradiated WI-38 human fibroblasts. *Proceedings of the Society for Experimental Biology and Medicine* **148**, 1249-1251.
- FURUKAWA, T., SAKUMA, S. & PLOTKIN, S. A. (1976). Human cytomegalovirus infection of WI-38 cells stimulates mitochondrial DNA synthesis. *Nature, London* **262**, 414-416.
- GORSKI, J., TOFT, D., SHYAMALA, G., SMITH, D. & NOTIDES, A. (1968). Hormone receptors: studies on the interaction of estrogen with the uterus. *Recent Progress in Hormone Research* **24**, 45-80.
- HIGGINS, S. J. & GEHRING, U. (1978). Molecular mechanisms of steroid hormone action. *Advances in Cancer Research* **28**, 313-397.
- HIRAI, K., FURUKAWA, T. & PLOTKIN, S. A. (1976). Induction of DNA polymerase in WI-38 and guinea pig cells infected with human cytomegalovirus (HCMV). *Virology* **70**, 251-255.
- JENSEN, E. V., SUZUKI, T., STUMPF, W. E., JUNGBLUT, P. W. & DESOMBRE, E. R. (1968). A two-step mechanism for the interaction of estradiol with rat uterus. *Proceedings of the National Academy of Sciences, U.S.A.* **59**, 632-638.
- JORDAN, M. C., SHANLEY, J. D. & STEVENS, J. G. (1977). Immunosuppression reactivates and disseminates latent murine cytomegalovirus. *Journal of General Virology* **37**, 419-423.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680-685.
- MICHELSON, S., HORODNICEANU, F., KRESS, M. & TARDY-PANIT, M. (1979). Human cytomegalovirus-induced immediate early antigens: analysis in sodium dodecyl sulfate-polyacrylamide gel electrophoresis after immunoprecipitation. *Journal of Virology* **32**, 259-267.
- MICHELSON-FISKE, S., HORODNICEANU, F. & GUILLON, J.-C. (1977). Immediate early antigens in human cytomegalovirus infected cells. *Nature, London* **270**, 615-617.
- MORHENN, V., RABINOWITZ, Z. & TOMKINS, G. (1973). Effects of adrenal glucocorticoids on polyoma virus replication. *Proceedings of the National Academy of Sciences, U.S.A.* **70**, 1088-1089.
- PARAN, M., GALLO, R. C., RICHARDSON, L. S. & WU, A. M. (1973). Adrenal corticosteroids enhance production of type-C virus induced by 5-iodo-2'-deoxyuridine from cultured mouse fibroblasts. *Proceedings of the National Academy of Sciences, U.S.A.* **70**, 2391-2395.

- PARKS, W. P. & SCOLNICK, E. M. (1974). Dexamethasone stimulation of murine mammary tumor virus expression: a tissue culture source of virus. *Science* **184**, 158-160.
- PENMAN, S., ROSBASH, M. & PENMAN, M. (1970). Messenger and heterogeneous nuclear RNA in HeLa cells: differential inhibition by cordycepin. *Proceedings of the National Academy of Sciences, U.S.A.* **67**, 1878-1885.
- PLUMMER, G., GOODHEART, C. R., HENSON, D. & BOWLING, C. P. (1969). A comparative study of the DNA density and behavior in tissue cultures of fourteen different herpes viruses. *Virology* **39**, 134-137.
- SAMUELS, H. H. & TOMKINS, G. M. (1970). Relation of steroid structure to enzyme induction in hepatoma tissue culture cells. *Journal of Molecular Biology* **52**, 57-74.
- SCOTT, W. A., SHIELDS, R. & TOMKINS, G. M. (1972). Mechanism of hormonal induction of tyrosine aminotransferase studied by measurement of the concentration of growing enzyme molecules. *Proceedings of the National Academy of Sciences, U.S.A.* **69**, 2937-2941.
- SMITH, M. G. (1959). The salivary gland viruses of man and animals (cytomegalic inclusion disease). *Progress in Medical Virology* **2**, 171-202.
- ST. JEOR, S. & RAPP, F. (1973). Cytomegalovirus: conversion of nonpermissive cells to a permissive state of virus replication. *Science* **181**, 1060-1061.
- ST. JEOR, S. C., ALBRECHT, T. B., FUNK, F. D. & RAPP, F. (1974). Stimulation of cellular DNA synthesis by human cytomegalovirus. *Journal of Virology* **13**, 353-362.
- STINSKI, M. F. (1976). Human cytomegalovirus: glycoproteins associated with virions and dense bodies. *Journal of Virology* **19**, 594-609.
- STINSKI, M. F. (1978). Sequence of protein synthesis in cells infected by human cytomegalovirus: early and late virus-induced polypeptides. *Journal of Virology* **26**, 686-701.
- TANAKA, S., FURUKAWA, T. & PLOTKIN, S. A. (1975). Human cytomegalovirus stimulates host cell RNA synthesis. *Journal of Virology* **15**, 297-304.
- TANAKA, J., YABUKI, Y. & HATANO, M. (1981). Evidence for early membrane antigens in cytomegalovirus-infected cells. *Journal of General Virology* **53**, 157-161.
- TANAKA, J., OGURA, T., KAMIYA, S., YOSHIE, T., YABUKI, Y. & HATANO, M. (1984). Dexamethasone enhances human cytomegalovirus replication human epithelial cell cultures. *Virology* (in press).
- WENTWORTH, B. B. & FRENCH, L. (1970). Plaque assay of cytomegalovirus strains of human origin. *Proceedings of the Society for Experimental Biology and Medicine* **135**, 253-259.
- YAMAMOTO, K. R. & ALBERTS, B. M. (1976). Steroid receptors: elements for modulation of eukaryotic transcription. *Annual Review of Biochemistry* **45**, 721-746.

(Received 9 January 1984)