

# Investigations on the Mechanism of Induction of the Alkaline Phosphatase by Bromodesoxyuridine in Herpes simplex Virus Transformed Cells and the Transport of Uridine

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Addition of BrdUrd in combination with prednisolone to HSV<sub>1</sub>-transformed hamster embryo cells induces an alkaline phosphatase (AP). FdUrd enhances, dThd reduces the inducing capacity of BrdUrd and prednisolone. Induction is prevented by addition of cycloheximide or of cytosine arabinoside.

BrdUrd reduces transport and phosphorylation of exogenously applied labeled Urd, especially the amount of UTP, UDP and Urd-diphosphate sugars. The Lineweaver-Burk-plot of uridine-uptake after addition of BrdUrd reveals the characteristics of the mixed type inhibition (competitive-noncompetitive).

## Introduction

In hamster tumor cells derived from hamster embryo fibroblasts transformed by herpes simplex virus (HSV) type 1 the plasma membrane bound alkaline phosphatase (AP) has nearly been lost, whereas an alkaline phosphodiesterase is still present [1]. Loss of AP activity also has been demonstrated after transformation of hamster embryo fibroblasts by Polyoma virus and SV<sub>40</sub> [2] and of chick embryo fibroblasts transformed by Rous sarcoma virus or Moloney sarcoma virus [3]. However, this loss does not seem to be a general phenomenon found after viral transformation *in vitro* [4]. AP negative transformed cells seem to have a selective advantage over comparable AP positive cells [2]. No decrease of AP activity was seen after lytic infection with different strains of HSV [5].

The AP is a rather nonspecific enzyme with many molecular forms and probably many functions [6]. The enzyme is known as a plasmamembrane marker [7]. Although the regulation of AP in animal cells, especially in HeLa cells, has been under investigation for many years the cellular role of this enzyme has not yet been determined. Results of Melnykovych *et al.* [8] and of Sela and Sachs [2] suggest that the enzyme may be associated with an intricate system of growth regulation. Furthermore, AP is well known for its inducibility in animal cells and there are many

reports describing its induction [9–14]. IdUrd, BrdUrd and hydrocortisone are the drugs mostly used for induction [15, 16]. AP activity seems to be regulated by glucocorticoids [17, 18]. By the two properties: Loss after viral transformation and inducibility, the AP looks as a good candidate to investigate other cell parameters. IdUrd is also used for induction of silent retrovirus genomes [19]. However, there is a lack of knowledge on the mechanism(s) of the inductive effect of IdUrd.

In this paper we report results concerning the induction of AP in hamster tumor cells transformed by HSV-1 by 5'-bromodeoxyuridine and prednisolone. The induction of AP after growth of the cells in medium containing BrdUrd suggests a change in the properties of the plasmamembrane. Therefore we investigated the uptake of uridine in BrdUrd treated hamster tumor cells. Since it is possible that BrdUrd induces additional effects besides AP induction which may influence the composition of the plasmamembrane, *e.g.*, by affecting the supply of nucleoside diphosphate sugars involved in membrane biosynthesis [20] the intracellular acid soluble metabolites derived from exogenous [<sup>3</sup>H]uridine in BrdUrd treated cells were compared with those of untreated cells.

## Materials and Methods

### Materials

Thymidine (dThd), deoxycytidine (dCyd), bromodeoxyuridine (BrdUrd), fluorodeoxyuridine

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(FdUrd), *p*-nitrophenylphosphate, prednisolone-succinate, naphthol AS-MX phosphoric acid (Na<sub>2</sub>-salt) were obtained from Sigma (München, Germany). Cycloheximide was a gift from the Upjohn Comp., Calamazoo, Michigan, USA). The uridine metabolites were purchased from Sigma Comp., München, Germany. [<sup>3</sup>H]uridine ([<sup>3</sup>H]Urd) was purchased from the Radiochemical Centre (Amersham, UK). The specific activity was 18–25 Ci/mmol. All solvents and substances for chromatography were purchased from Merck (Darmstadt, Germany). Cell culture bottles were from Nunc (Wiesbaden, Germany). Calf serum was purchased from Gibco. Chromatographic paper 2043 a was from Schleicher & Schüll (Dassel, Germany).

#### Cell cultivation

Hamster tumor cells were derived from tumors induced by s. c. injection of Herpes simplex virus (type 1) transformed hamster embryo fibroblasts into weanling hamsters as described [1]. Transformation was performed by UV-irradiated HSV-1. These cells were obtained by Dr. L. Thiry, Brussels and were injected s. c. at the backside of 3 days old syrian hamsters (10<sup>6</sup> cells in 0.1 ml PBS (animal)). Tumors developed regularly in 100% of the inoculated animals within 2–3 weeks. After excision of the tumors, cell cultures could be easily established. The cells were seeded at a density of 2×10<sup>6</sup> cells/bottle in 81 cm<sup>2</sup> Nunc cell culture bottles and grown in Eagle's Basal Medium containing 10% calf serum, penicillin (200 units/ml) and streptomycin (20 units/ml).

#### Chemical determinations

Protein was determined after the method of Lowry *et al.* [21], DNA after the method of Burton [22].

#### Determination of alkaline phosphatase

AP activity was determined by a standard colorimetric procedure using 8 mM *p*-nitrophenylphosphate in 0.5 M 2-amino-2methyl-1propanol · HCl buffer pH 10.5 with 10<sup>-3</sup> M MgCl<sub>2</sub> [23].

If not otherwise stated, cells were homogenized by ultrasonication in 0.1 M Na-barbiturate · HCl buffer pH 8.2 containing 1% deoxycholate. Aliquots of the homogenate were mixed with the assay solution in a quartz cuvette and the release of *p*-nitrophenylate

was followed in a Zeiss PM QII spectrophotometer at 410 nm. The total assay volume was 1.0 ml and 1 unit of AP activity is defined as the formation of 1 nmol *p*-nitrophenol per mg protein per hour. The enzyme is strongly inhibited by levamisole – HCL (Sigma, München; Germany).

#### Presentation of data

All results reported are representative of at least three independent experiments, and each determination within an experiment as an average of three replicate flasks.

The lines were fitted to the points by the method of least squares.

#### Chromatography of ethanole soluble radioactivity

The cell pellet harvested as described below was extracted four times with 1 ml of ice-cold 50% ethanole containing 10% phosphate buffered saline [24].

The combined extracts were diluted with water and lyophilized. These samples were resuspended in water and subjected to paper chromatography.

Descending paper chromatography was performed with Schleicher and Schüll 2043 a chromatographic paper in solvent equilibrated chambers at room temperature for 48 h. Resolution of UTP, UDP, UMP and UDP-glucose (UDP-Glc) from each other was in solvent A (95% ethanole: 1 M ammonium acetate pH 5.0; 70:30 v/v) with poor separation of UMP and UDP-N-acetyl-glucosamine (UDP-NAcGlcNH<sub>2</sub>).

Resolution of UMP from UDP-NAcGlcNH<sub>2</sub>, UDP-Glc and UTP, UDP with poor separation of UTP and UDP was seen in solvent B (95% ethanole: 1 M ammonium acetate pH 7.5; 5:2 v/v). Mixtures of appropriate standards were co-chromatographed.

After chromatography the positions of the marker nucleotides were localized under UV light. Chromatograms were cut at right angles to the direction of migration into 1 cm×3 cm segments and counted directly in Bray's solution in a tricarb liquid scintillation spectrometer.

#### Measurement of uridine uptake

In essence, the experiments were performed as described earlier [3]. For uptake studies the growth medium (25 ml) was replaced by 5 ml of fresh

medium. 10 min after medium renewal cells were pulsed-labeled with [ $^3\text{H}$ ]Urd at the indicated concentrations.

Following incubation at 37 °C for the indicated times, medium containing radioisotope was quickly aspirated, and cultures were rapidly washed by sequential (3 times) addition and aspiration of ice-cold phosphate buffered saline. After washing the cells were harvested by scraping off into 3 ml of ice-cold phosphate buffered saline and centrifugation for 10 min at 8000  $\times g$ .

For determination of acid-soluble and acid-insoluble radioactivity the cell pellet was extracted with 4  $\times$  1 ml of ice-cold 10% (w/v) trichloroacetic acid and the extracts sampled for acid-soluble radioactivity. The last extract contained only 50 to 100 dis/min/1 ml. The trichloroacetic acid insoluble sediment was dissolved in 0.5 M NaOH at 37 °C.

Aliquots of the the TCA-soluble fraction and the TCA-insoluble fraction dissolved in NaOH were removed for counting radioactivity in Bray's scintillation mixture [25] in a Tricarb liquid scintillation spectrometer (Packard, model 3330).

Efficiency corrections to take account for quenching were performed by the external standard ratio method using a tritiated source.

Aliquots of the NaOH dissolved TCA-insoluble fraction were removed to determine DNA and protein content.

Kinetic studies of uptake of [ $^3\text{H}$ ]Urd were done by applying different volumes of a mixture of [ $^3\text{H}$ ]Urd and nonlabeled Urd to the cell cultures giving a constant specific activity at a constant volume of 10 ml medium.

After washing and harvesting the cells in the described manner uptake of Urd was estimated by determining the rate or incorporation into total cell material as done earlier [26].

## Results

### Induction of AP activity

Hamster tumor cells derived from tumors induced by injection of HSV (type 1) transformed hamster embryo fibroblasts in weanling hamsters contain only a minimal activity of AP [1]. Initial experiments (not shown here) showed that BrdUrd in concentrations from 5  $\mu\text{g}$  to 15  $\mu\text{g}$  per ml induces the activity of the AP to the same degree. We therefore used 10  $\mu\text{g}$  per ml of BrdUrd to study the induction of the enzyme.

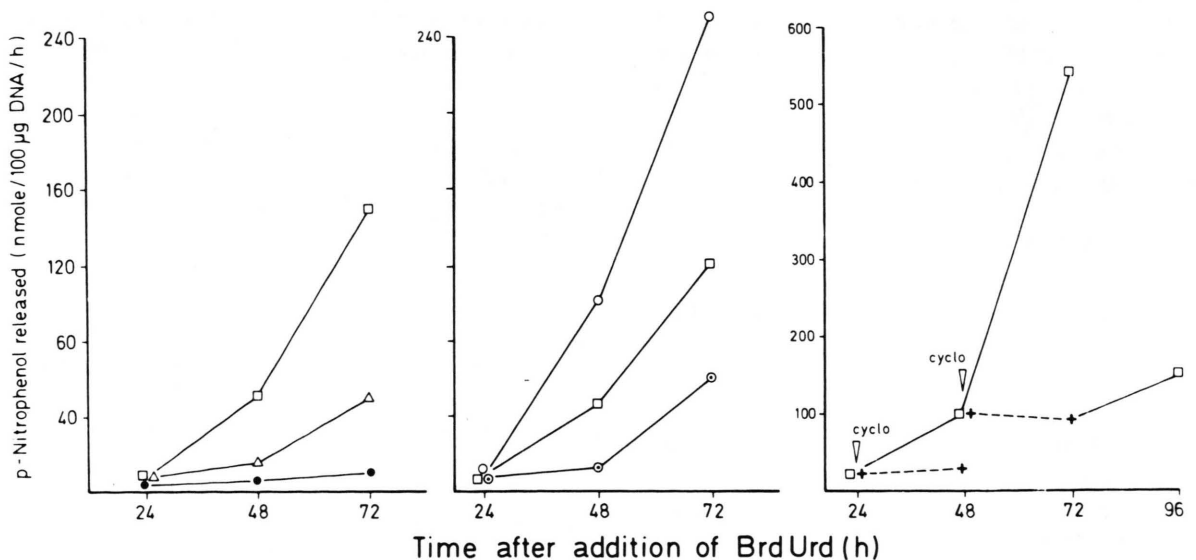


Fig. 1. a) Induction of AP activity by BrdUrd or BrdUrd and/or prednisolone. The drugs were added at zerotime and the activity of the AP was determined 24, 48 and 72 h later. □ BrdUrd and prednisolone; △ BrdUrd; ● prednisolone. b) Influence of FdUrd (0.8  $\mu\text{g}/\text{ml}$ ) or of dThd (10  $\mu\text{g}/\text{ml}$ ) on the combined induction capacity of 10  $\mu\text{g}/\text{ml}$  of BrdUrd and 5  $\mu\text{g}/\text{ml}$  of prednisolone. The cells were treated as described in Fig. 1 a. □ BrdUrd and prednisolone; ○ BrdUrd plus additional FdUrd; ◐ BrdUrd plus additional dThd. c) Addition of 5  $\mu\text{g}/\text{ml}$  of cycloheximide (arrow ↓; x---x) after induction of AP by BrdUrd and prednisolone. □ BrdUrd and prednisolone.

As can be seen from Fig. 1 a there is no significant stimulation of enzyme activity up to 24 h after addition of the drug. At 48 h the activity of the AP was increased to a level 3 times of control values and reaches a level of about 10 times that in the controls at 72 h.

We were also interested whether prednisolone (5 µg/ml) induces AP activity. As can be seen also from Fig. 1 a there is a negligible induction of AP by prednisolone during the 24–72 h growth period.

However, combined addition of BrdUrd and prednisolone resulted in a strong increase of the enzyme activity: At 72 h the activity of AP increases to a level about 30 times that of the untreated controls and about 3 times that of the cells treated with BrdUrd alone.

The growth of cells in medium containing BrdUrd or BrdUrd and prednisolone, respectively, has no significant influence on the DNA or protein content of the cells as can be judged from Table I.

#### *Effect of dThd and FdUrd on AP induction by BrdUrd and prednisolone*

Incorporation of BrdUrd into DNA has been suggested to be responsible for the mode of action of the drug [27]. An approach to test this hypothesis is to study the effect of drugs which influence the degree of BrdUrd incorporation in cells with normal DNA synthesis. The ability of dThd to prevent the incorporation of BrdUrd into DNA by direct competition for phosphorylation [28] has been used by different workers to study the relationship of BrdUrd effects, *e.g.* virus activation, to the degree of its incorporation [29]. FdUrd enhances the incorporation of BrdUrd into DNA by depleting the cell of thymidylate [30].

In the following experiments were described concerning the influence of dThd and FdUrd on the

induction of AP by BrdUrd alone and by BrdUrd combined with prednisolone.

As shown in Fig. 1 b treatment of hamster tumor cells with BrdUrd and prednisolone together with 10 µg dThd reduces the activity of AP to about 27% at 48 h and about 52% at 72 h, respectively, compared to that of BrdUrd and prednisolone treated cells. Treatment of tumor cells with 0.8 µg/ml FdUrd together with 10 µg/ml BrdUrd and 5 µg/ml prednisolone resulted in a 52% increase of AP activity after 72 h compared to the value of cells treated with BrdUrd and prednisolone alone.

#### *Inhibition of protein synthesis and induction*

Cycloheximide was added to BrdUrd and prednisolone treated cells 24 h after addition of the inducers.

As can be seen from Fig. 1 c the addition of this inhibitor of protein synthesis also inhibits the induction of AP.

Addition of cycloheximide to cell cultures growth for 24 or 48 h in BrdUrd and prednisolone caused an immediate cessation of the increase of AP activity. Removal of the inhibitor at 72 h by changing the medium to a medium containing only BrdUrd and prednisolone reverses the inhibition of protein synthesis and also the inhibition of the increase of AP activity.

#### *Uptake of [<sup>3</sup>H]Urd into BrdUrd treated cells*

In hamster tumor cells derived from HSV-1 transformed hamster embryo fibroblasts AP cannot be detected in the plasmamembrane [1]. The AP induced by BrdUrd and BrdUrd and prednisolone was mainly found in the plasmamembrane fraction [31]. Therefore it was tempting to assume that the appearance of this new plasmamembrane component

Table I. DNA- and protein-content of Hamster embryo tumor cells transformed by HSV-1 at different times after treatment with BrdUrd or BrdUrd and prednisolone.

Time [h]	Control		+ BrdUrd		+ BrdUrd + prednisolone	
	DNA [µg]	Protein [mg]	DNA [µg]	Protein [mg]	DNA [µg]	Protein [mg]
24	680 ± 40*	12.5 ± 0.6	785 ± 40	14.3 ± 0.7	710 ± 40	13.3 ± 0.6
48	1290 ± 70	23.9 ± 1.2	1200 ± 60	21.6 ± 1.0	1230 ± 60	22.5 ± 1.1
72	1520 ± 75	24.4 ± 1.2	1680 ± 90	26.0 ± 1.3	1580 ± 80	24.4 ± 1.1

BrdUrd 10 µg/ml; prednisolone 5 µg/ml.

\* Standard deviation.



could possibly exercise certain influences on the uptake of metabolic precursors. In the following, data are presented concerning the uptake of Urd into hamster tumor cells grown in the presence of BrdUrd.

Concerning transport of Urd the rate of permeation through the membrane has been suggested to be the rate limiting process [32] but also it has been repeatedly suggested that phosphorylation may play an integral part in the uptake process [33–37].

In this report “uptake” of Urd refers to the total quantity of radioactivity derived from [ $^3\text{H}$ ]Urd found to be cell-associated (acid soluble and acid insoluble) after incubation of the cell with the nucleoside under defined conditions.

Tumor cells grown in the presence of 10  $\mu\text{g}/\text{ml}$  BrdUrd for 24, 48 and 72 h were puls labeled with [ $^3\text{H}$ ]Urd, and acid soluble and acid insoluble radioactivity was determined (Table II). The uptake increases in the control cell from 24 to 48 h. This may well be depend on the stage of the growth cycle of the cells. As can be seen there is no obvious difference in the uptake of [ $^3\text{H}$ ]Urd between cells grown for 24 h in the presence of BrdUrd and control cells.

At 48 h acid soluble radioactivity has decreased to 33% and acid insoluble radioactivity to 50% giving an decrease of about 39% for total radioactivity. These values did not change significantly after 72 h.

The rate of Urd uptake at various substrate concentrations over a 10 min period was measured in

Table II. Acid soluble and acid insoluble tritium-labeled [ $^3\text{H}$ ]uridine in BrdUrd treated and control cells after 24, 48 and 72 h.

Time [h]	Acid-soluble *		Acid-insoluble *	
	Control	BrdUrd	Control	BrdUrd
24	7400 $\pm$ 600 *	6800 $\pm$ 600	1000 $\pm$ 50	900 $\pm$ 46
48	12000 $\pm$ 1000	4000 $\pm$ 400	6000 $\pm$ 530	3000 $\pm$ 270
72	14000 $\pm$ 1200	4200 $\pm$ 400	6800 $\pm$ 600	3300 $\pm$ 300

dpm/10  $\mu\text{g}$  DNA  $\times 10^3$ ; BrdUrd 10  $\mu\text{g}/\text{ml}$ ; puls labeling was done for 20 min.

\* Standard deviation.

untreated and BrdUrd treated cells after 48 h of growth and the uptake velocity plotted versus the substrate concentration, using the reciprocal Lineweaver-Burk-plot [38]. Uptake of Urd in untreated hamster tumor cells followed as Fig. 2 indicates normal Michaelis-Menten-kinetic with an apparent  $K_m$  of 8  $\mu\text{M}$ . This value is in agreement with  $K_m$ 's for Urd transport in other cell systems [32].

However, Urd uptake in BrdUrd treated cells is quite different. The uptake in this system is characterized by a higher  $K_m$  (18  $\mu\text{M}$ ) as well as a lower  $V_{\text{max}}$  compared to the untreated cell. In the Lineweaver-Burk-plot a mixed type of inhibitions results.

#### *Analysis of intracellular metabolites derived from [ $^3\text{H}$ ]Urd*

Separation of total ethanole extracts by paper chromatography of hamster tumor cells grown in the

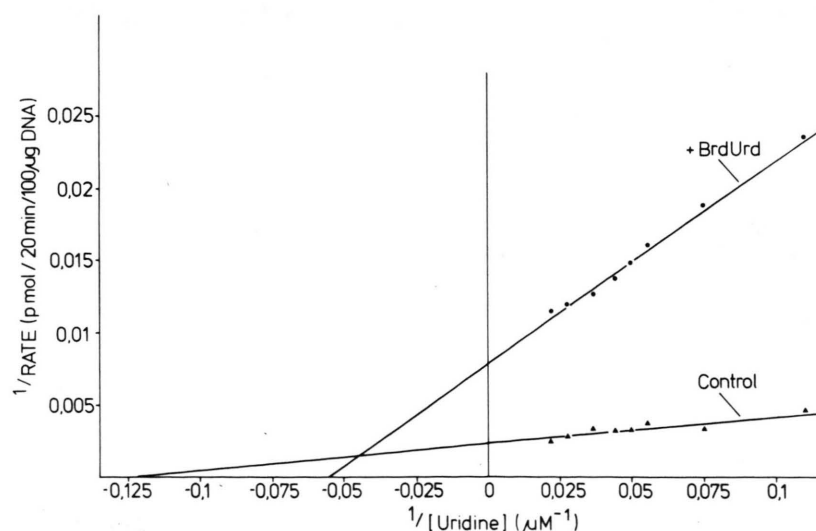


Fig. 2. Lineweaver-Burk-plot of uptake of Urd of cells 48 h after addition of BrdUrd.

Table III. Chromatography of ethanole-extracts from cells labelled with [<sup>3</sup>H]uridine and grown in the presence and absence of BrdUrd.

Time [h]		Total <sup>3</sup> H [dpm]	per cent of total <sup>3</sup> H				
			UTP	UDP	UDP-Glc	UMP + UDP-NAcGlcNH <sub>2</sub>	Urd
24	Control	5075	47	17	18	14	5
	BrdUrd	4400	43	18	19	16	3
48	Control	18000	25	30	19	22	3
	BrdUrd	15000	16	27	21	31	5
72	Control	24000	21	23	26	28	3
	BrdUrd	16300	7	12	18	45	18

Chromatography system A; puls labeling was done for 10 min; UDP-Glc = UDP-glucose; UDP-NAcGlcNH<sub>2</sub> = UDP-N-Acetylglucosamine.

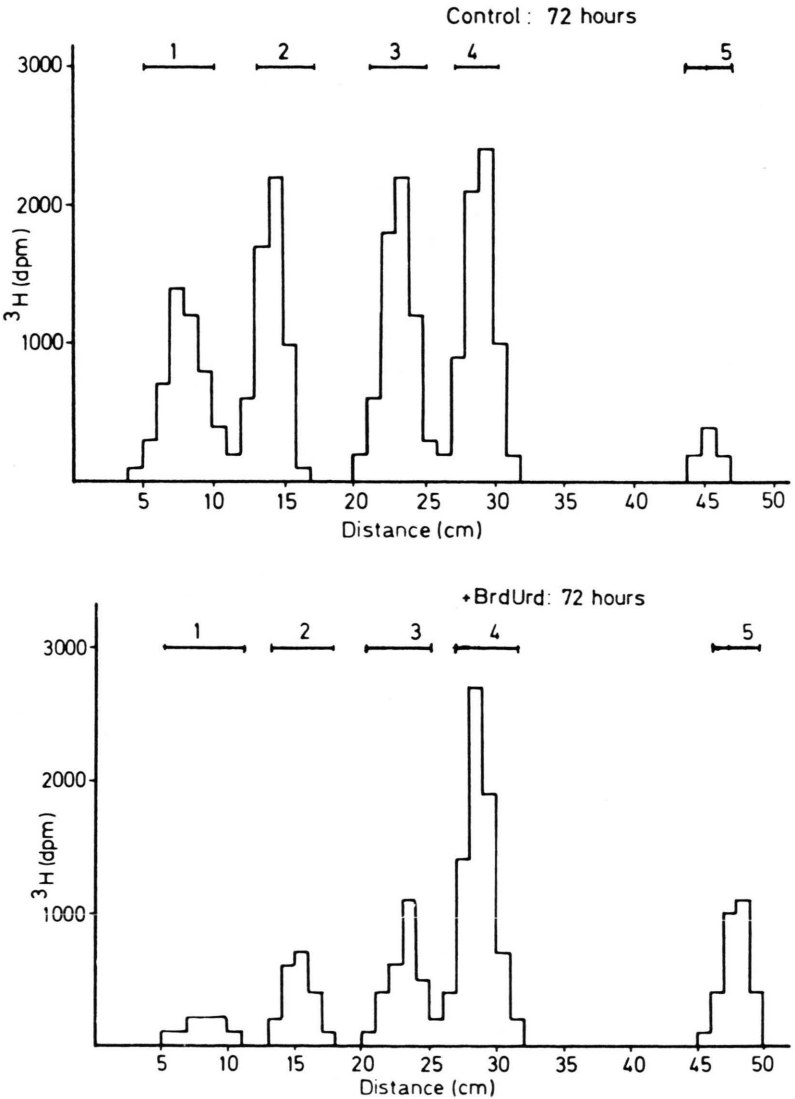


Fig. 3. Separation of intracellular Urd phosphorylation products by the chromatography system A at 72 h. The bars refer to UTP (1), UDP (2), UDP-Glc (3), UMP and UDP-NAcGlcNH<sub>2</sub> (4) and Urd (5).

Table IV. Chromatography of ethanol-extracts from cells labeled with [ $^3$ H]Urd and grown in the presence and absence of BrdUrd.

Time [h]		Total $^3\text{H}$ [dpm]	Per cent of total $^3\text{H}$				
			UTP + UDP	UMP	UDP-Glc	UDP-NAcGlcNH <sub>2</sub>	Urd
72	Control	20800	44	17	24	12	3
	+ BrdUrd	13000	25	35	15	7	17

Chromatography system B; puls labeling was done for 10 min; UDP-Glc = UDP-glucose; UDP-NAcGlcNH<sub>2</sub> = UDP-N-Acetylglucosamine.

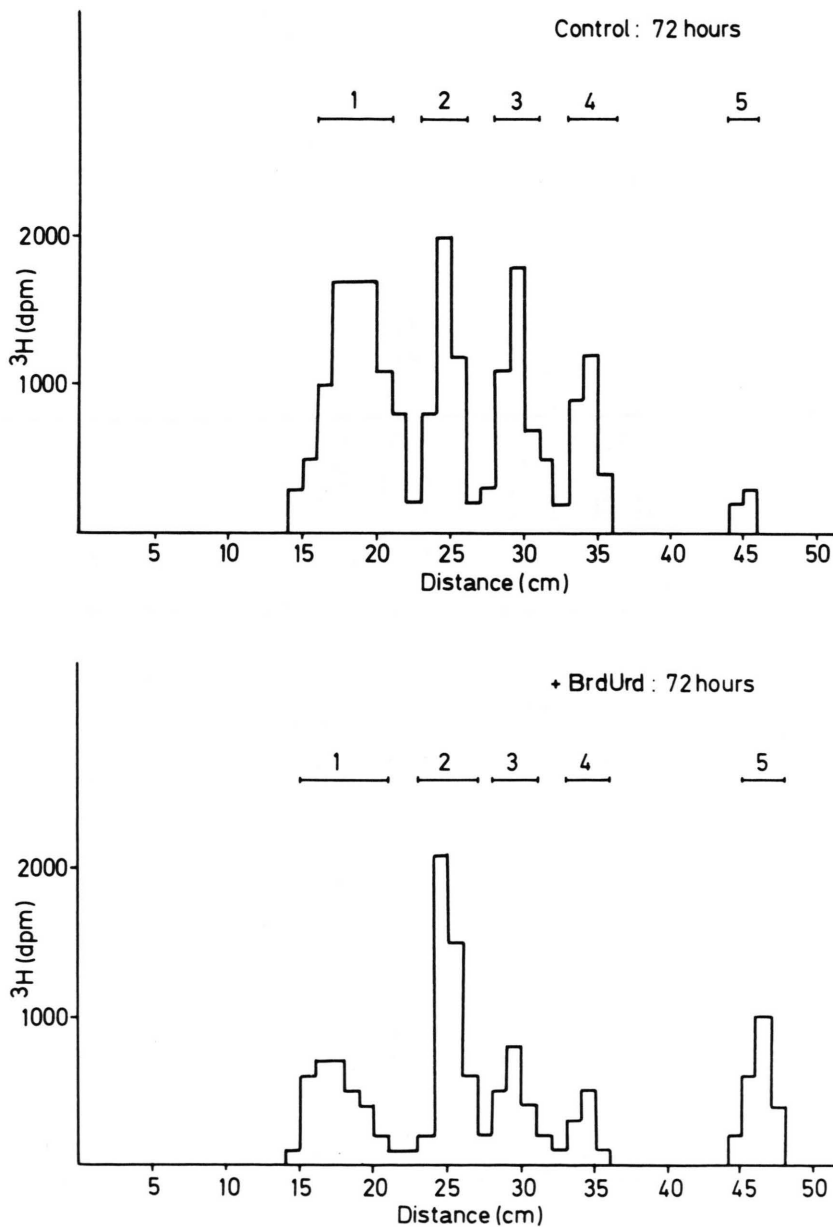


Fig. 4. Separation of intracellular Urd phosphorylation products by the chromatography system B at 72 h. The bars refer to UTP and UDP (1), UMP (2), UDP-Glc (3), UDP-NAcGlcNH<sub>2</sub> (4) and Urd (5).

absence or presence of BrdUrd and puls labeled with [ $^3\text{H}$ ]Urd revealed peaks which comigrated with the markers UTP, UDP, UMP, UDP-Glc, UDP-NAcGlcNH<sub>2</sub> and Urd (Fig. 3, Table III).

As can be seen from Table III (chromatographic system A) growth of cells in BrdUrd medium for 24 h does not significantly change the incorporation of radioactivity into nucleotides with the exception of UTP which shows a slight reduction of incorporation (91%, relative to control value as 100%).

At 48 h an obvious decrease of radioactivity comigrating with UTP (64% of control) and an increase of that migrating in the region of UMP/UDP-NAcGlcNH<sub>2</sub> (141%, relative to control value as 100%) is observed.

At 72 h, radioactivity associated with UTP has further decreased to 33% of control values and UMP/UDP-NAcGlcNH<sub>2</sub> associated radioactivity increased to 161% of controls. At 72 h also a significant reduction of radioactivity associated with UDP (to 52%) and UDP-Glc (to 69%) is found, together with a strong increase of radioactivity migrating in the region of Urd.

To differentiate whether the strong increase of radioactivity associated with UMP/UDP-NAcGlcNH<sub>2</sub> is due to an increase of incorporation of the label into UMP and/or UDP-NAcGlcNH<sub>2</sub> we used paper chromatography system B which shows better separation of UMP and UDP-NAcGlcNH<sub>2</sub> (Fig. 4, Table IV).

As Fig. 4 and Table IV show the total increase of radioactivity at 72 h associated with UMP/UDP-NAcGlcNH<sub>2</sub> in system A is due to an increase of radioactive UMP (206%) whereas the labeled UDP-NAcGlcNH<sub>2</sub> is reduced to about 58% of the control in system B.

From these results it is likely to conclude that the decrease in utilization of exogenous [ $^3\text{H}$ ]Urd merely reflects an influence of BrdUrd treatment on the phosphorylation of Urd via UMP to UTP and may not be primarily a result of decreased uptake of Urd into the cell. The main reason for this assumption is that at 48 and 72 h the uptake of acid soluble radioactivity in BrdUrd treated cells is about the same.

## Discussion

Our results show that BrdUrd increases the activity of the AP in hamster embryo cells transformed by HSV-1 to about 10-fold, whereas the addition of

prednisolone is without effect. The combined addition of both drugs however, leads to a 30-fold increase of this enzymatic activity. Differences have been observed earlier on the inducing capacity of various inducers.

In HeLa cells AP may be increased up to about 20-fold by addition of 1  $\mu\text{M}$  prednisolone to the medium [39]. Goz and Walker [40] reported that in HeLa cells IdUrd as well as prednisolone alone can induce AP to a similar degree. Using IdUrd and prednisolone together they found that at 24 and 48 h, the specific activity in cells treated with both compounds was about the sum of the activities in cells in which both compounds were used separately. At 72 and 96 h the activity from cells treated with IdUrd and prednisolone together was less than the sum of activities in cells treated with either agent alone, but higher than the single activities.

Concerning the mechanism of this induction process we showed that tritium-labeled BrdUrd is incorporated into cellular DNA (data not presented). Its amount is reduced by addition of cold dThd. Addition of cold dThd also reduces the degree of induction of AP. On the other hand, addition of FdUrd to the system increases the amount of the induced enzyme.

Experiments with cytosine arabinoside (not shown here) a well known inhibitor of cellular DNA synthesis [41] point also in the direction that DNA synthesis and BrdUrd incorporation into DNA are prerequisites for the induction of AP, because AP activity does not increase when cytosine arabinoside is present during the BrdUrd treatment. Cytosine arabinoside does not inhibit the ribonucleotide reductase complex [42, 43]; however there may be other unknown mechanisms of the action of cytosine arabinoside.

It could be shown also that protein-synthesis is necessary for the induction process: Addition of cycloheximide inhibited the inductive effect of BrdUrd and prednisolone.

From the experiments with these drugs which have different modes of action it is tempting to conclude that a procedure which enhances the incorporation of BrdUrd into DNA increases the activity of the induced enzyme. When DNA-synthesis and protein synthesis are inhibited no induction of AP can be observed.

The observation that protein synthesis is necessary for induction of AP is similar to results reported for



induction of AP by IdUrd in HeLa cells [40]. Goz and Walker suggest two interpretations of this effect. Either IdUrd may increase AP activity by inducing the synthesis of an AP-modifying protein. In this case the inhibition of protein synthesis prevents a further increase of the AP enhancing activity. On the other hand, only newly synthesized AP can be modified to increase enzyme activity. Inhibiting protein synthesis prevents further supply of newly synthesized AP. Possibly, both interpretations may be true for the inhibition of AP activity increase in hamster tumor cells after induction, but their verification demands further experiments.

The addition of BrdUrd in combination with prednisolone to HSV-1 transformed hamster embryo cells not only induces AP activity but also influences the Urd metabolism. Accordingly, the phosphorylation of Urd in cells is reduced considerably at 48 h after addition of BrdUrd. It attracts attention that the striking decrease in Urd uptake is observed after 48 h at the very moment when significant induction of AP activity is demonstrable. From the coincidence of decrease of Urd uptake and increase in AP activity together with the lag phase of about 48 h it is tempting to conclude that the decrease of Urd uptake is the consequence of certain specific meta-

bolic changes in the cell, at least correlated in time with the increase of AP activity.

Furthermore, under our conditions, the transport kinetics of Urd at 48 h is altered: The  $K_m$  value increases from 8  $\mu$ M to 18  $\mu$ M; this is correlated to a decrease of the  $V_{max}$ . The mixed type of inhibition of the Urd-transport in the Lineweaver-Burk-plot indicates not only quantitative but also qualitative changes of the activity of the Urd-transport system. Similar observations have been made earlier concerning the inhibition kinetics of the choline kinase activity by Cpd 48/80 [44].

Analysis of the soluble products of the phosphorylation of Urd in BrdUrd treated cells show a reduced rate of phosphorylation of Urd. Interestingly, besides UTP and UDP also the amount of Urd-diphosphate sugars is reduced. This may indicate a faulty and insufficient synthesis of precursors for cell membrane biosynthesis, especially the glycosylation of proteins. This may be perhaps directly connected with the altered Urd-transport system.

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