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The Effect of Lithium Chloride on the Replication of *Herpes simplex* Virus

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Abstract. Lithium chloride inhibited the replication of type 1 and type 2 *Herpes simplex* virus at concentrations which permitted host cell replication. Virus polypeptide and antigen synthesis were unaffected while viral DNA synthesis was inhibited. The replication of two other DNA viruses, pseudorabies and vaccinia virus, was inhibited but there was no inhibition of two RNA viruses, namely, EMC and influenza virus.

Introduction

Lithium salts were first introduced into medical practice for the treatment of 'gout and rheumatic gout' (Garrod, 1859) where the high solubility of lithium urate was expected to promote uric acid secretion. The Extra Pharmacopoeia of 1908 indicates further therapeutic indications for lithium salts including 'Bright's disease', epilepsy, syphilis, and, again, 'chronic gout and rheumatism'. There was no evidence of therapeutic efficacy in any of these conditions and by the late nineteen-forties, lithium therapy was not only out of vogue but in some disfavour following three case reports of lithium intoxication in subjects using lithium chloride as a salt substitute (Corcoran et al., 1949; Hanlon et al., 1949; Stern, 1949).

It was curious, therefore, that, in that same year (1949), Cade demonstrated the anti-manic effect of the lithium ion which was confirmed in later studies by Schou et al., (1954). There now seems little doubt of the therapeutic value of lithium salts in the treatment of acute mania and in the prophylaxis of depressive episodes (Hartigan, 1963; Baastrup et al., 1970; Kline, 1968).

The effect of lithium on the behaviour and replication of microorganisms has received little attention. Hadley et al. (1931) reported that incorporation of 70 mM lithium chloride into beef infusion broth encouraged the generation of filterable G forms of *Shiga* bacillus and, more recently, Morishita and Tokada (1975) and Heiniss et al. (1977) reported that while lithium had a 'sodium-sparing' effect for the growth

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of *Vibrio Parahaemolyticus*, higher concentrations in excess of 300 mM inhibited growth of the organism. To our knowledge, the effect of lithium salts on virus replication has not been investigated, although high concentrations of lithium iodide (6M) have been employed in the physical separation of viral DNA from capsid and core proteins (Neurath et al., 1970).

This study investigates the effect of lithium chloride on the replication of several DNA and RNA viruses. Virus particle production, polypeptide and antigen synthesis and virus DNA synthesis are examined for type 1 and 2 *Herpes simplex* virus.

Materials and Methods

Cells

Baby hamster kidney cells (BHK 21 cells, McPherson and Stoker, 1962) were used for production of virus stocks, virus titrations, and for experiments investigating the effect of lithium chloride on virus replication. The cells were maintained on supplemented Eagle's medium (Vantsis and Wildy, 1962).

Virus Strains

The following viruses were tested; type 2 *Herpes simplex* strain '3345' and type 1 *Herpes simplex* strain 'HF' (Plummer et al., 1974), the Dekking strain of pseudorabies virus, the Lister strain of vaccinia virus, the NWS strain of influenza A virus, a standard laboratory strain of adenovirus type 2 and encephalomyocarditis virus.

General Experimental Method

Subconfluent monolayers of BHK 21 cells were prepared in 5-cm diameter disposable plastic petri dishes. The cells were infected at a multiplicity of 10 plaque-forming units (pfu) per cell by incubation under 1 ml of an appropriate dilution of virus suspension for 1 h at 37°C. The cells were then washed three times with pre-warmed ETC and finally re-incubated at 37°C in 4 ml of ETC medium containing the required concentration of lithium chloride. Infected cultures were disrupted by ultrasonic vibration and virus yields titrated in BHK 21 cells by the plaque method of Russell (1962). Encephalomyocarditis virus and influenza A, NWS strain, were titrated on preformed monolayers under a 0.5% agarose overlay. The dilution of infected cell extracts at which plaques were counted was always of sufficient magnitude to ensure that there was no effect from residual lithium chloride in the virus titration system. Herpes particles were counted by negative staining, using 2% phosphotungstic acid, against an index of polystyrene latex beads (Agar Aids; Watson et al., 1964). Soluble virus antigens were investigated in Ouchterlony immunodiffusion gels using high quality rabbit hyperimmune herpes simplex virus antisera prepared as described by Watson et al. (1966). Complement-fixing antigen titers were investigated as previously described (Skinner et al., 1976).

Herpes simplex Virus Polypeptide Synthesis

Virus-infected and uninfected cell monolayers were incubated at 37°C for 3 h in medium containing the required concentrations of lithium chloride. The monolayers were

washed three times with warm amino-acid-free Eagle's medium containing 2% calf serum and the polypeptides labelled by addition of ^{35}S-methionine at 3 μCi per dish in methionine-free Eagle's medium. After 15-h incubation in the required lithium concentration, the medium was removed, the monolayers washed twice with Dulbecco A, and the cells harvested in 1 ml of disruption mixture, 2% SDS 5% 2-mercapto-ethanol, 0.05 M tris-hydrochloride, pH 7.0, 3% sucrose, and 0.005% bromophenol blue at 80°C. The samples were heated at 80°C for 10 min., disrupted with an ultrasonic probe (MSE 100-watt ultrasonic disintegrator) and stored at -70°C.

The polypeptides were separated by polyacrylamide slab gel electrophoresis and identified autoradiographically as described by Harper et al., (1979).

Herpes simplex Virus DNA Synthesis

Cells were infected with 10 pfu/cell as above. At 4 1/2 h after infection the medium was removed and the cells incubated with Eagle's medium containing appropriate concentrations of lithium chloride, 5% calf serum, and ^{3}H-thymidine at a final concentration of 10 μCi/ml. After a further 2 h the cells were rinsed in phosphate-buffered saline and extracted into 1 ml DNA buffer (0.05 M tris, 1 mM EDTA, 1 mM mercapto-ethanol pH 8.5: Mantyjarvi and Russell, 1969) containing 2% sodium lauryl sarcosine (Sigma Chemical Co. Ltd.). The samples were heated at 60°C for 10 min and sheared by pipetting.

Samples of 0-1ml were loaded on to 4.2 ml caesium chloride (density 1.706 gm/ml), in DNA buffer and centrifuged at 40,000 rpm for 3 days at 20°C in an MSE 10 x 10 ml fixed-angle rotor (Flamm et al., 1966). 0.2 ml fractions were collected and the radioactivity precipitable by 5% trichloroacetic acid was determined. The refractive index of selected fractions was measured and the density calculated.

Chemical Reagents

Anhydrous lithium chloride and crystalline lithium sulphate, sodium chloride and potassium chloride were obtained from BDH Chemicals Ltd. More concentrated stock solutions of lithium chloride in supplemented Eagle's medium were stored at 4°C for up to 3 weeks without loss of potency.

Results

Herpes simplex Viruses

Effect on Virus Infectivity. The replication of *Herpes simplex* virus in the presence of varying concentrations of lithium chloride is shown in Fig. 1. Virus replication measured at 24 h was reduced at drug concentrations of 5 mM and completely inhibited by concentrations in excess of 30 mM. The effect was specific to the lithium ion as similar inhibition of virus replication was obtained with equivalent molarities of lithium sulphate while no inhibition was obtained with equivalent molarities of sodium and potassium chloride.

The effect of 60 mM lithium chloride on virus replication at various stages in the growth cycle is shown in Fig. 2. Virus replication was inhibited if the drug was added during the first 6 h of infection with no significant inhibition of replication when the drug was added at later times of infection.

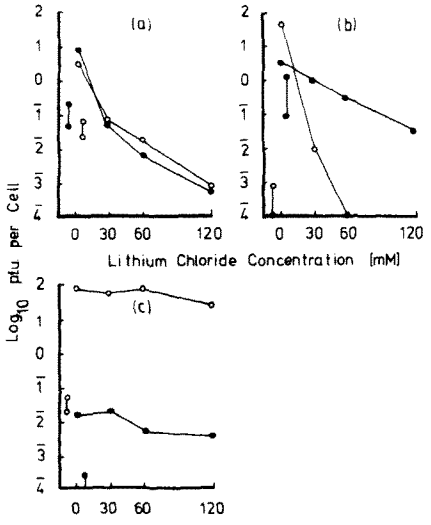

Fig. 1. The effect of varying concentrations of lithium chloride on the replication of DNA and RNA viruses. The upper symbol on the vertical bar indicates the $\log_{10}$ virus yield per cell following the adsorption period and the lower symbol, the $\log_{10}$ virus yield per cell at 4 h of infection.

(a) Type 1 *Herpes simplex* virus ■-■
Type 2 *Herpes simplex* virus □-□
(b) Pseudorabies virus □-□
Vaccinia virus ■-■
(c) Influenza virus ■-■
Encephalomyocarditis virus □-□

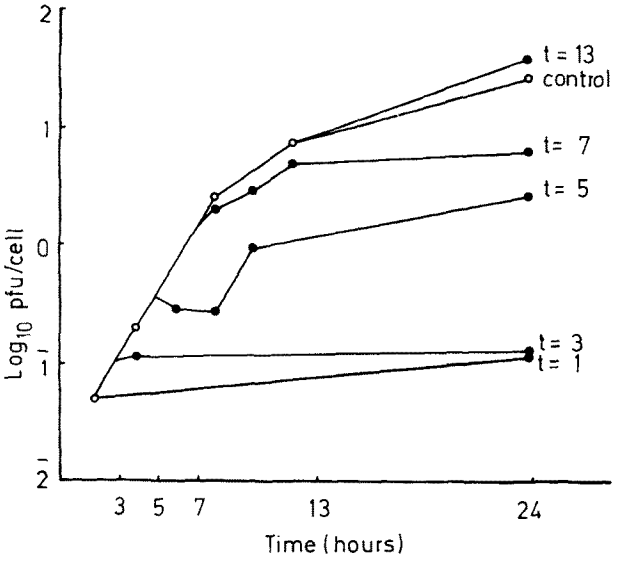

Fig. 2. The effect of adding 60 mM lithium chloride at various hours ('*t*') following infection of cells wtih type 1 *Herpes simplex* virus

Effect of Virus Particle Production. An 80 oz roller bottle of sub-confluent cells (containing approximately 2 x 10^8 cells) was infected with 0.5 pfu per cell of type 1 *Herpes simplex* virus. Inoculum virus was removed by threefold washing with phosphate-buffered saline, incubation for 1 h in 10% pooled human serum followed by re-washing of the monolayers three times with phosphate-buffered saline to remove residual human serum. In the presence of 60 mM lithium chloride, there was no evidence of virus

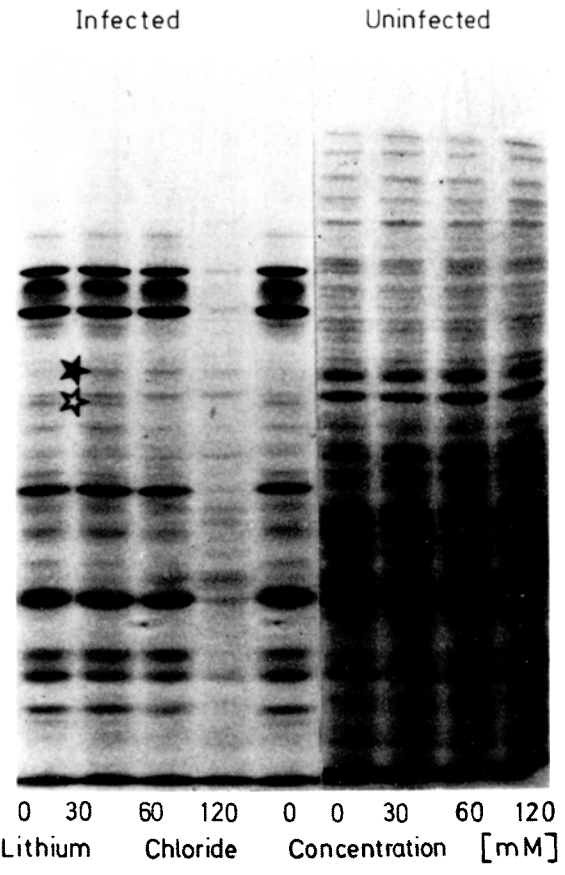

Fig. 3. The effect of varying concentrations of lithium chloride on virus polypeptide synthesis

particle production at 24 h after infection; indeed, virus infectivity and particle production were lower than obtained following 2 h of infection in the absence of lithium. Similar results were obtained with type 2 herpes virus.

Effect on Virus Polypeptide Synthesis. Drug concentrations of 60 mM permitted synthesis of most virus polypeptides (Fig. 3). The bands corresponding to certain polypeptides were decreased in intensity (☆ in Fig.) and one polypeptide (★ in Fig.) seemed to present in greater intensity at 60 mM lithium chloride than at lower (30 mM) or zero drug concentrations. There was little, if any, virus polypeptide synthesis at 120 mM of lithium chloride.

Effect on Virus Antigen Synthesis. There was normal representation of immunoprecipitating virus antigens at 60 mM lithium chloride but no immunoprecipitating antigens were detectable at a concentration of 120 mM.

Virus DNA Synthesis. Synthesis of type 2 *Herpes virus* DNA was completely inhibited by concentrations of 30 mM lithium chloride and partially depressed by concentrations of 7.5 mM (Fig. 4).

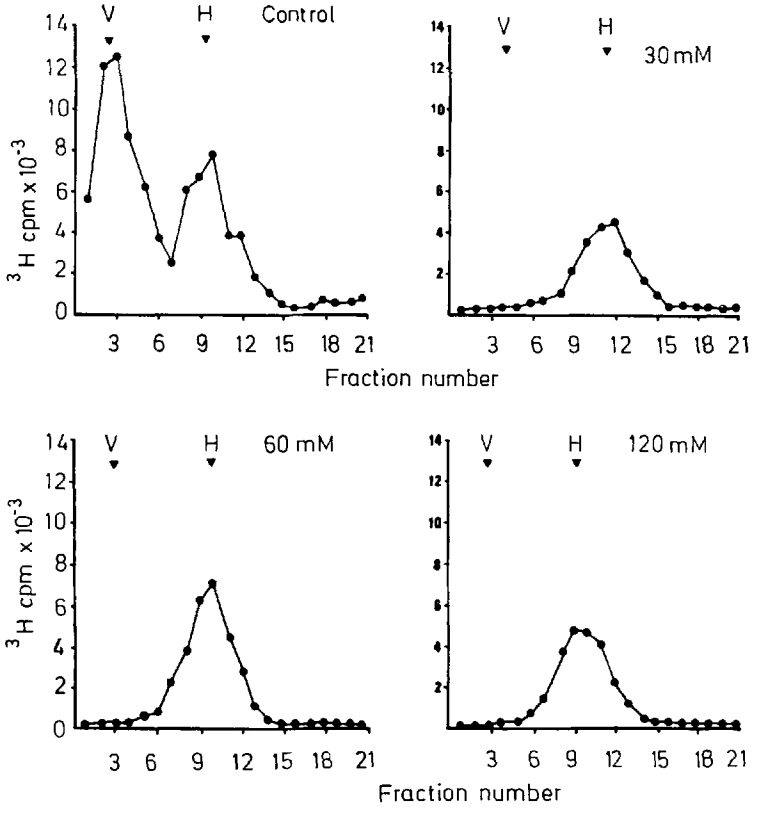

Fig. 4. The effect of varying concentrations of lithium chloride on the synthesis of type 2 *Herpes simplex* virus DNA. Virus DNA V; Host cell DNA H

Effect on Other Viruses. The replication of two other DNA viruses, pseudorabies, a herpes virus, and vaccinia virus, a pox virus, was inhibited (Fig. 1); preliminary results indicate that adenovirus type 2 was also inhibited by similar drug concentrations. On the other hand, there was no significant inhibition of replication of EMC or influenza A virus, strain NWS (Fig. 1).

Effect on Host Cells. BHK-21 cells replicated in medium containing 30 mM lithium chloride but would complete only one cycle of replication at 60 mM concentration of the drug. At 40 mM drug concentration, there was a reduced rate of cell replication. Drug effects were easily reversible. After incubation of cells in medium containing 120 mM lithium chloride for 72 h, normal cell growth continued following re-incubation of the cells in lithium-free medium.

Discussion

This study has demonstrated inhibition of replication of *Herpes simplex* and other DNA viruses by lithium chloride at concentrations which permit host cell replication

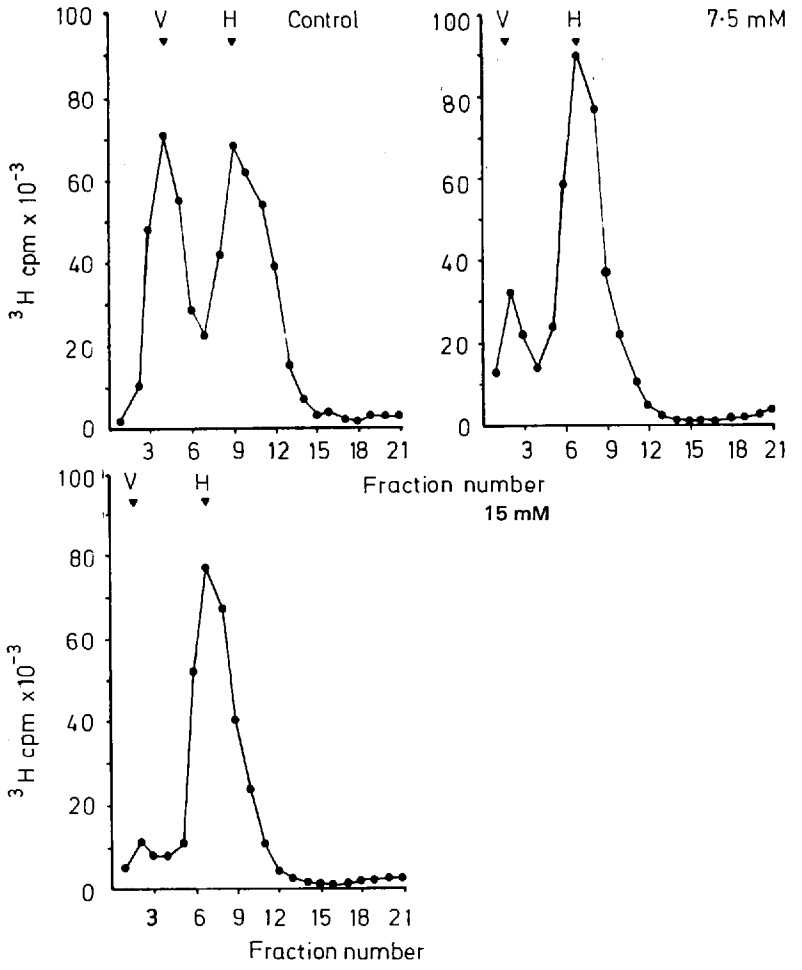

Fig. 4. (cont.)

and normal replication of two RNA viruses, namely, EMC and influenza virus. Lithium chloride would appear to act by inhibiting DNA synthesis. This effect accords in general with studies in mouse mammary epithelial cells which were blocked in the G1 phase by 50 mM lithium chloride (Turkington, 1968) and with our own study where BHK 21 cells completed one cycle of replication in 60 mM lithium. Herpes virus DNA synthesis is more sensitive than BHK cell DNA synthesis to inhibition by various concentrations of lithium chloride (Fig. 4). This relative inhibition of virus over host cell DNA synthesis merits further investigation in other virus-cell systems and in cell-free nucleic acid replicative systems.

Preliminary results using an in vitro enzyme assay have indicated that DNA polymerase activity in uninfected BHK cells is inhibited by 90% in the presence of 50 mM lithium chloride. Similar inhibitions have been noted for rat liver tissue (Howk and

Wang, 1969), kidney tissue (Lazarus and Kitron, 1974), calf thymus (Bollum, 1962) and Walker tumour cells (Furlong, 1965) and the effect was more pronounced than that obtained with other monovalent cations: sodium, potassium, and ammonium. DNA polymerase activity from *Herpes* virus-infected cells, however, was not inhibited in vitro by 60 mM lithium chloride although a partial inhibition (50%) of DNA polymarase enzyme synthesis in cells inoculated in 60 mM of the drug was observed. This level of inhibition could not, however, account for the virtually complete cessation of virus DNA synthesis and further experimentation is necessary to determine the precise mode of action of lithium salts in this context. The results suggest, however, that lithium chloride may have application as an investigative tool in the mechanisms of cell transformation by DNA viruses of the herpes, adeno-; and papovavirus group and of the retroviruses where the mechanism of transformation is mediated via a double-stranded DNA intermediate.

The drug merits trial as a local agent for cutaneous or muco-cutaneous infections of herpetic, adenoviral, or vaccinial etiology where the continued production of virus antigens by virus-infected cells in the presence of lithium ensures an immunogenic offering to the infected host. The relative predilection of the drug for brain tissue (Francis and Traill, 1970; Morrison et al., 1971) and the facility with which the drug enters the cerebro-spinal fluid (Hanlon et al., 1949; Gershon, 1970) suggest a possible application in certain noncutaneous DNA virus infections, for example, herpetic or vaccinial encephalitis or possibly the long-term treatment of progressive multifocal leucoencephalopathy of papovavirus etiology. It is interesting, moreover, that a recent study indicated that lymphocyte and macrophage functions were enhanced in vitro by concentrations of only 1-5 mM lithium chloride (Shenkman et al., 1978). On the other hand, systemic therapy would, presumably, require a degree of selective drug concentration in the appropriate target organ as standard lithium dose regimes achieve serum concentrations of 0.7 - 1.3 mM which will inhibit virus replication by only a small extent in our in vitro system (Fig. 1).

A final application of the drug concerns a possible role in DNA virus vaccine production. The drug inhibits virus particle and virus DNA synthesis, but permits normal virus antigen synthesis; this may be convenient for the preparation of a non-infectious and non-oncogenic virus vaccine and it is anticipated that this approach may supersede our present method of herpes virus vaccine production (Skinner et al., 1978) and provide a simple, safe, and economic method of vaccine production for herpes and other DNA viruses.

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