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Effect of a Nuclear Polyhedrosis

Virus of the Spruce Budworm on

the Metabolic Processes of Vertebrate Cells

by

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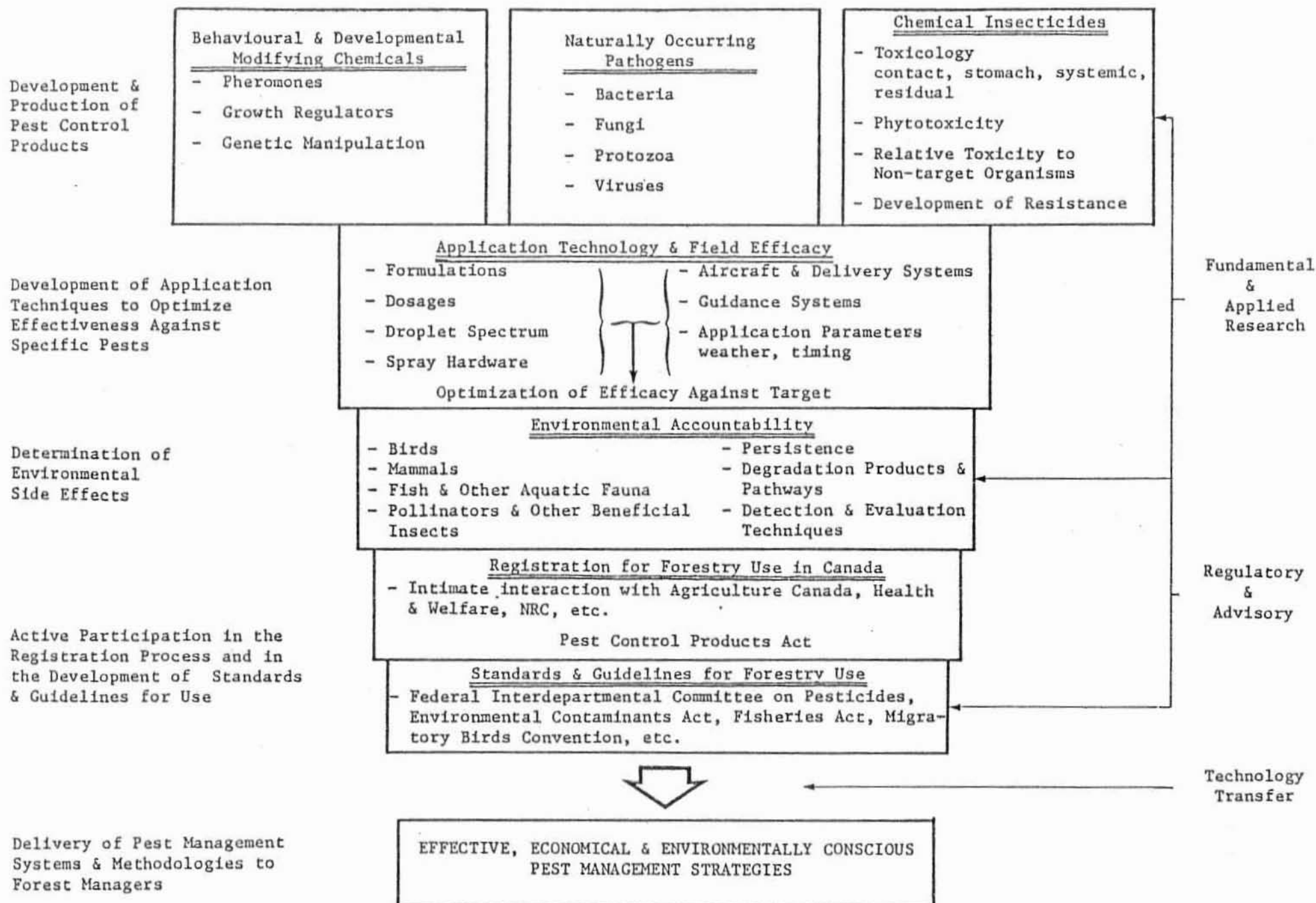
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Environment Canada

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Development & Production of Pest Control Products

Behavioural & Developmental Modifying Chemicals
- Pheromones
- Growth Regulators
- Genetic Manipulation

Naturally Occurring Pathogens
- Bacteria
- Fungi
- Protozoa
- Viruses

Chemical Insecticides
- Toxicology
  contact, stomach, systemic, residual
- Phytotoxicity
- Relative Toxicity to Non-target Organisms
- Development of Resistance

Development of Application Techniques to Optimize Effectiveness Against Specific Pests

Fundamental & Applied Research

Application Technology & Field Efficacy
- Formulations
- Dosages
- Droplet Spectrum
- Spray Hardware
- Aircraft & Delivery Systems
- Guidance Systems
- Application Parameters weather, timing

Optimization of Efficacy Against Target

Determination of Environmental Side Effects

Environmental Accountability
- Birds
- Mammals
- Fish & Other Aquatic Fauna
- Pollinators & Other Beneficial Insects
- Persistence
- Degradation Products & Pathways
- Detection & Evaluation Techniques

Active Participation in the Registration Process and in the Development of Standards & Guidelines for Use

Regulatory & Advisory

Registration for Forestry Use in Canada
- Intimate interaction with Agriculture Canada, Health & Welfare, NRC, etc.

Pest Control Products Act

Standards & Guidelines for Forestry Use
- Federal Interdepartmental Committee on Pesticides, Environmental Contaminants Act, Fisheries Act, Migratory Birds Convention, etc.

Delivery of Pest Management Systems & Methodologies to Forest Managers

Technology Transfer

EFFECTIVE, ECONOMICAL & ENVIRONMENTALLY CONSCIOUS PEST MANAGEMENT STRATEGIES

ABSTRACT

Mouse, fish and bird cell cultures were inoculated with a nuclear polyhedrosis virus of the spruce budworm, Choristoneura fumiferana, and at one, two or three days after inoculation radioactive uridine, thymidine or leucine was added. At 2-h intervals thereafter, the cells were processed and the synthesis of RNA, DNA or protein was determined. The results, compared to those from mock infected cells, were presented as qualitative incorporation of the radioactive precursor into the respective macromolecule. The data showed that the synthesis of RNA, DNA or protein increases linearly with time and there was no difference between infected and mock infected cells observed.

RÉSUMÉ

L'auteur inocula des cellules de Vertebres (Souris, Poisson, Oiseau) avec un virus de la polyhédrose nucleaire de la Tordeuse des Bourgeons de l'Epinette, Choristoneura fumiferana, puis un, deux ou trois jours après l'inoculation, il ajouta de l'uridine, de la thymidine ou de la leucine radioactives. Ensuite, à intervales de deux heures, les cellules furent travaillées et l'auteur effectua la synthèse de l'ARN, l'ADN ou la protéine. Il présente les résultats, comparés à ceux de cellules pseudo-infectées, en tant qu'une incorporation qualitative du précurseur radioactif dans chaque macromolécule respective. Il observe que la synthèse de l'ARN, l'ADN ou la protéine augmente linéairement avec le temps et que nulle différence observable n'existe entre les cellules vraiment et faussement infectées.

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INTRODUCTION

The spruce budworm, Choristoneura fumiferana, is one of the most serious forest pest in Eastern Canada and the North Eastern United States. Every year this insect is responsible for the destruction of large areas of spruce-fir forest. So far, only chemical insecticides have been used on a widescale basis to control infestations. However, the chemical insecticides currently used are ecologically unacceptable since they affect many non-target organisms. On the other hand, microbial insecticides generally affect only the target insect and appear to be safe for vertebrates.

A nuclear polyhedrosis virus (NPV) of the spruce budworm has been used experimentally as a biological insecticide and to date, over 1600 ha of infested forest have been sprayed with this virus. However, before widespread use of this NPV as a registered forest control product its effect on non-target organisms must be assessed.

In collaboration with the Ontario Veterinary College at the University of Guelph safety tests were undertaken to ascertain the pathogenicity of spruce budworm NPV to mammals and birds, (1,2). After extensive testing the results proved conclusively that this insect virus neither multiplies in vertebrates nor does it seem to alter their metabolic processes. The effect of NPV on the metabolism of vertebrates can be elucidated more definitively and accurately by inoculating cells grown in vitro with virus and studying macromolecular synthesis in the presence of radioactive precursors. In this report the effect of NPV on the qualitative biosynthesis of nucleic acids and proteins is investigated.

MATERIALS AND METHODS

Virus

Virions were released from purified inclusion bodies by alkali treatment and were then subjected to rate zonal and equilibrium centrifugation on sucrose gradients (3). Each preparation was bioassayed in fifth or sixth instar larvae and all virion preparations were infectious.

Cells

The cells to be tested were L cells (mouse epithelial), chick embryo fibroblasts (CEF), RTG-2 (trout cells) and fat headed minnow cells (FHM). They were grown on the bottom of clean, sterile glass scintillation vials in complete minimum essential medium (MEM) at the appropriate temperature until confluent monolayers were obtained. One half of the cultures were infected with NPV (6-10 µg per culture flask) and the other half were mock infected. The virus was allowed to adsorb for two hours. At one, two and three days after infection 2 ml of medium, containing radioactive precursor, was added to six infected and six uninfected cultures.

Radioactive Labeling

(a) In order to label intracellular proteins, leucine deficient medium was used to which ^{3}H-leucine was added to contain 5µCi/ml. Leucine defficient medium consisted of 5% (v/v) of complete MEM (without calf serum) and 95% leucine free MEM. To this mixture 2% dialyzed

calf serum was added followed by the addition of ^{3}H-leucine.

(b) To label intracellular RNA, serum free MEM containing 5μCi/ml of ^{3}H-uridine, was added to the cell cultures.

(c) For DNA labeling the cells were seeded at lower densities to give semi-confluent monolayers in 1-2 days. This ensured active DNA synthesis even after 3 days of incubation. The cells were labeled with ^{3}H-thymidine (7μCi/ml) in serum free MEM.

Infected and uninfected cultures were analyzed for acid pre-cipitable radioactivity on days 1, 2 and 3 at 2-h intervals over a period of 12 h. The radioactive medium was removed and the cells were rinsed three times with cold phosphate buffered saline (PBS), three times with ethanol and twice with ether. The precipitated and fixed monolayers at the bottom of the vials were dried at room temperature, 3 ml of scintillation cocktail was added and the amount of incorporated radioactivity was assayed in a liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Protein Synthesis

L cells were inoculated with virion and separate cultures were maintained at 28°C and 37°C. ^{3}H-leucine was added at 1,2 and 3 days after virus inoculation and the cells were examined for acid precipitable radioactivity at 2-h intervals thereafter. Mock infected cells were processed similarly (Fig. 1). The data presented in this figure show that virus inoculated cells had the same rate of protein synthesis as mock infected cells. Even 3 days after infection the rate of protein biosynthesis remained the same. These data show that NPV has no effect on mammalian cellular protein synthesis.

The cells were maintained at 28°C because it is the optimum temperature for NPV multiplication in the host insect. At 28°C the level of protein synthesis is lower than that at 37°C which is expected since 28°C is considerably below the optimum temperature for growth of mammalian cells (Fig. 1).

Similar data were obtained when using RTG-2 and FHM cells (Fig. 2). RTG-2 cells cannot grow at temperatures much higher than 22°C and FHM cells cannot grow at temperatures much lower than 28°C so tests were conducted only at these temperatures.

Experiments were carried out for only 2 days at 32°C and 37°C using CEF cells, since these cells begin to deteriorate after this time (Fig. 3).
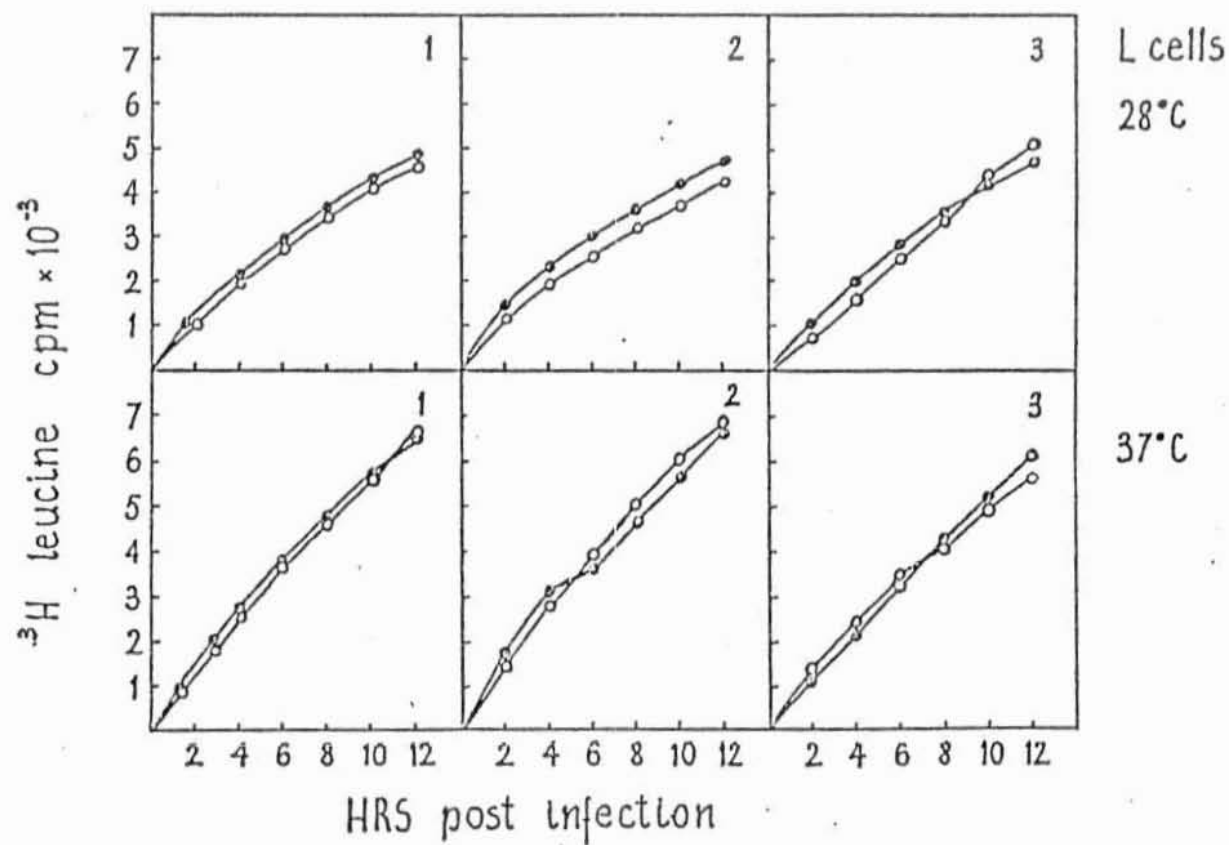
Figure 1. Rate of protein synthesis in L cells inoculated with NPV (●) and in uninoculated (o) cells. Days 1, 2 and 3.
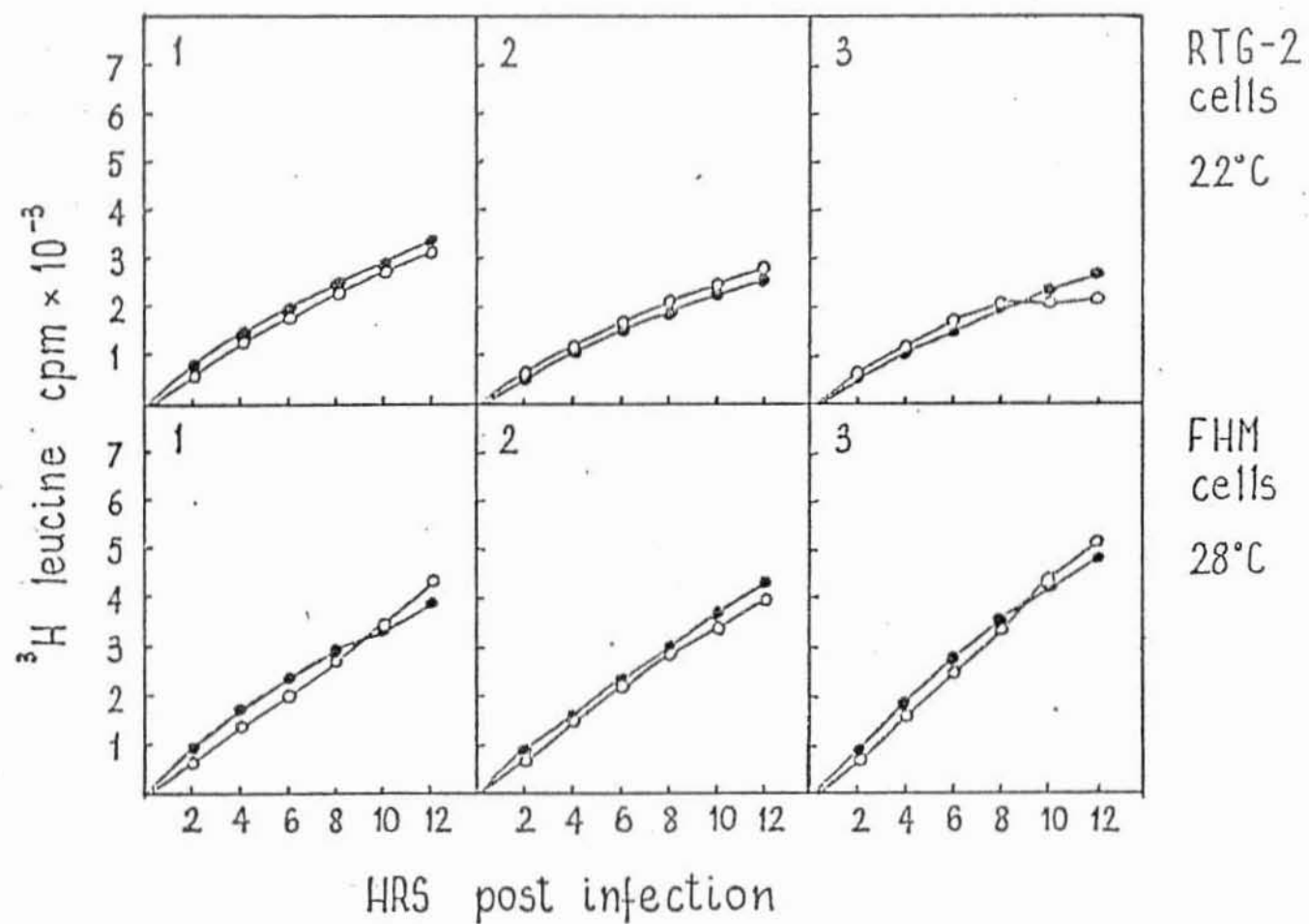
Figure 2. Rate of protein synthesis in RTG-2 and FHM cells. NPV inoculated cells (●) and uninoculated cells (o). Days 1, 2 and 3.
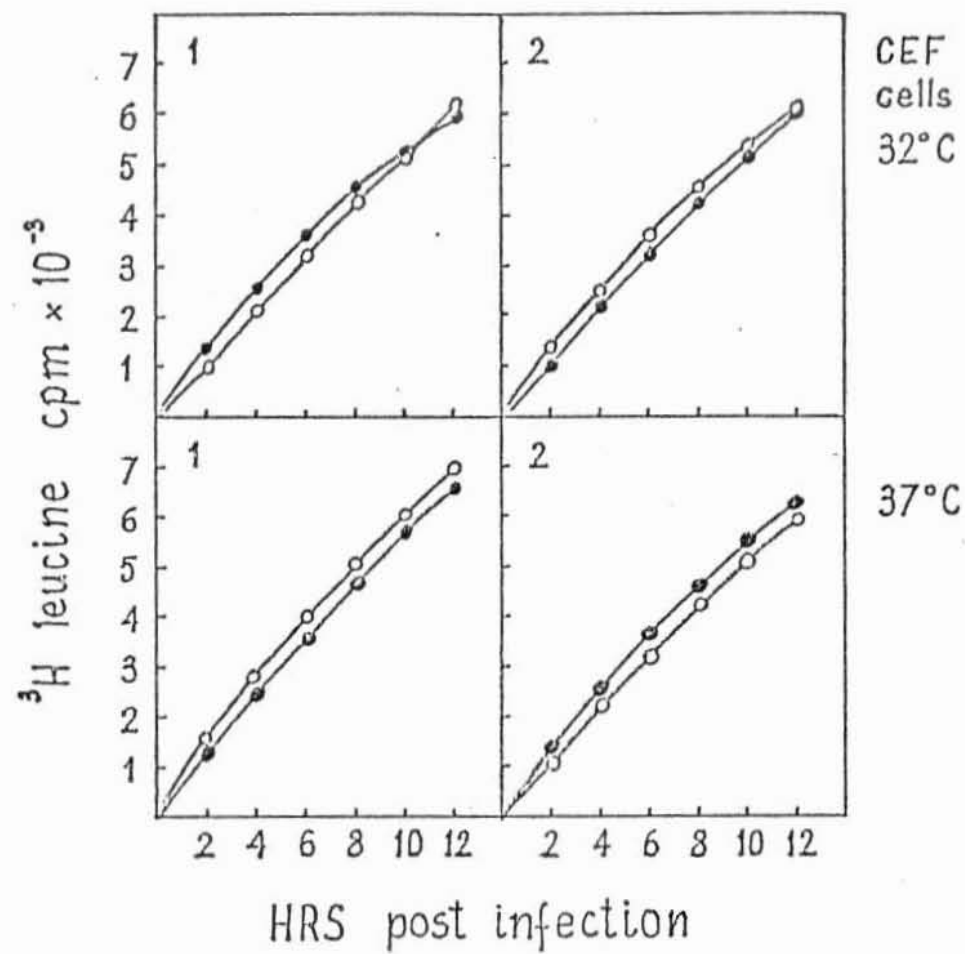
Figure 3. Rate of protein synthesis in CEF cells. NPV inoculated cells (●) and uninoculated cells (o). Days 1 and 2.

It is interesting to note that the rate of protein biosynthesis in CEF cells was unaltered when the cultures were maintained at 28°C or 37°C. The results summarized in Fig. 2 and 3 show that NPV had no inhibitory or altering effect on avian or fish cell protein synthesis.

The above data demonstrate that these cells are refractory to NPV as far as qualitative protein synthesis is concerned. Any effect on the ribosomes, cellular mRNA or tRNA would have been manifested in an altered rate of protein biosynthesis which was not observed.

RNA Synthesis

The experiments were similar to those used for monitoring the rate of protein synthesis except that ^{3}H-uridine, a specific RNA metabolic precursor, was used. L cells grown at 37°C and 28°C exhibited no difference in the rate of RNA synthesis between infected and mock infected cultures (Fig. 4). It is clear, however, that the rate of RNA synthesis at 28°C is much slower than that at 37°C since the latter is the optimum temperature for vertebrate cells. Similar data were obtained for CEF cells (Fig. 5) and for the two fish cell lines (Fig. 6).

It is well documented that a number of viruses inhibit protein and RNA synthesis in infected cultures (4). It is also clear that NPV had no effect whatsoever on the quantitative RNA synthesis of these four cell lines even at a temperature close to the optimum for NPV multiplication.
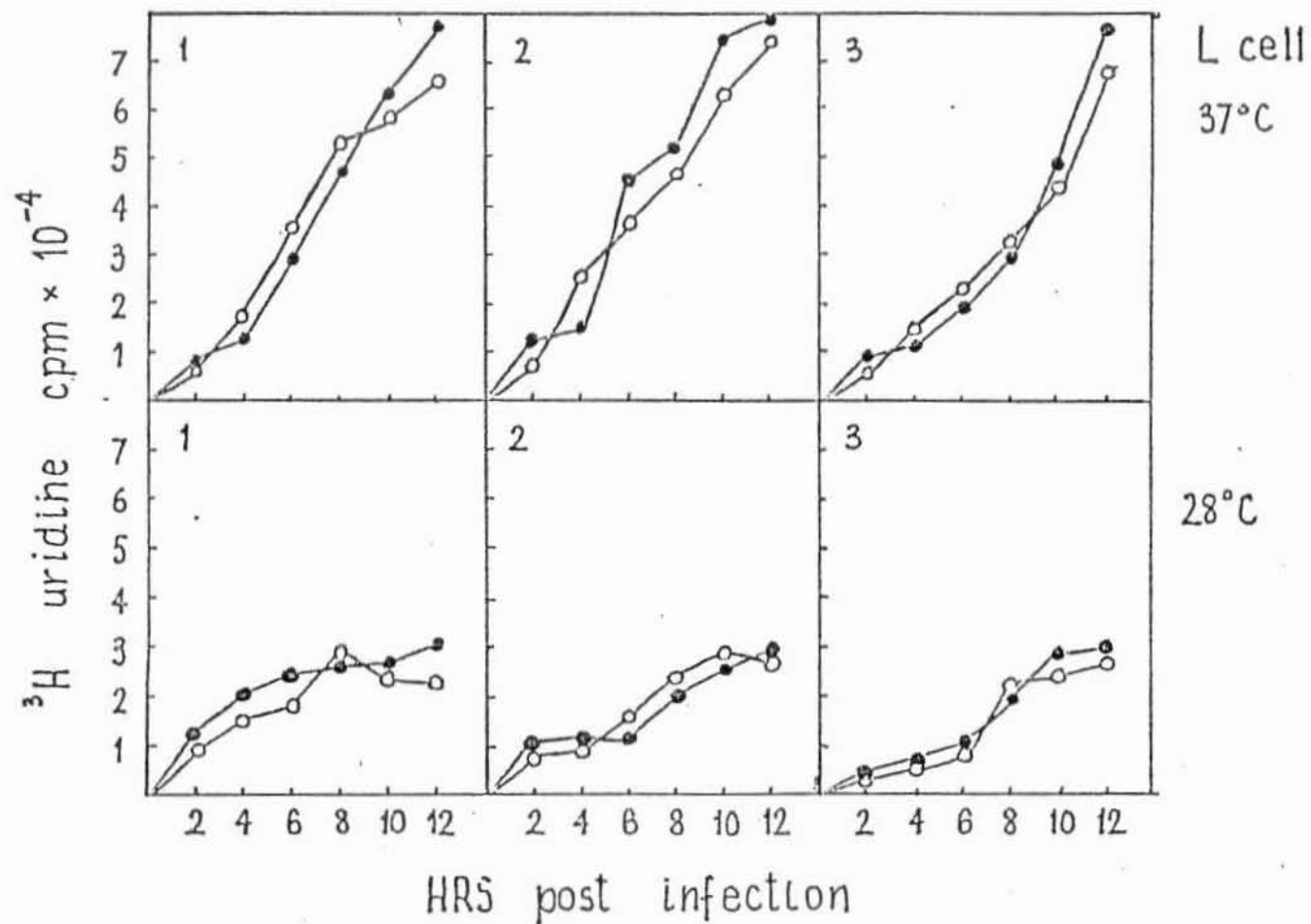
Figure 4. RNA synthesis in L cells incubated at 37°C and at 28°C. NPV inoculated cells (●) and mock infected cells (o). Days 1, 2 and 3.
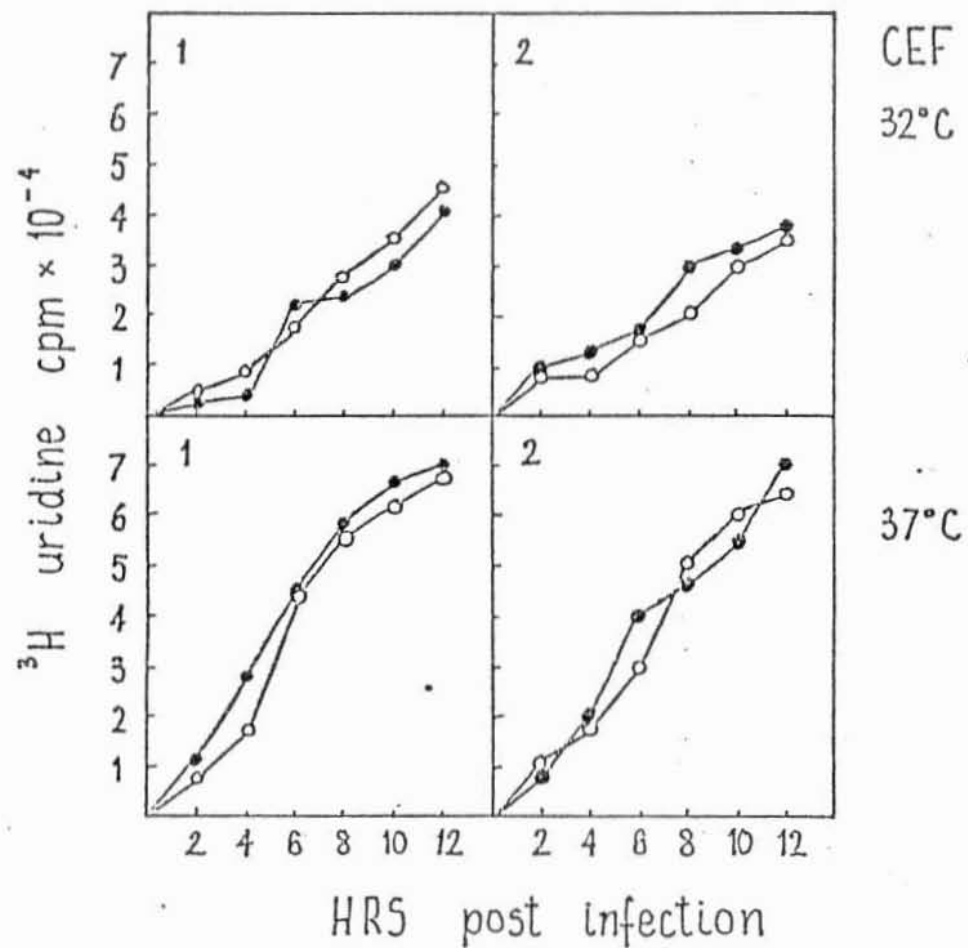
Figure 5. RNA synthesis in CEF cells at 32°C and at 37°C. NPV inoculated cells (●) and mock infected cells (o). Days 1 and 2.
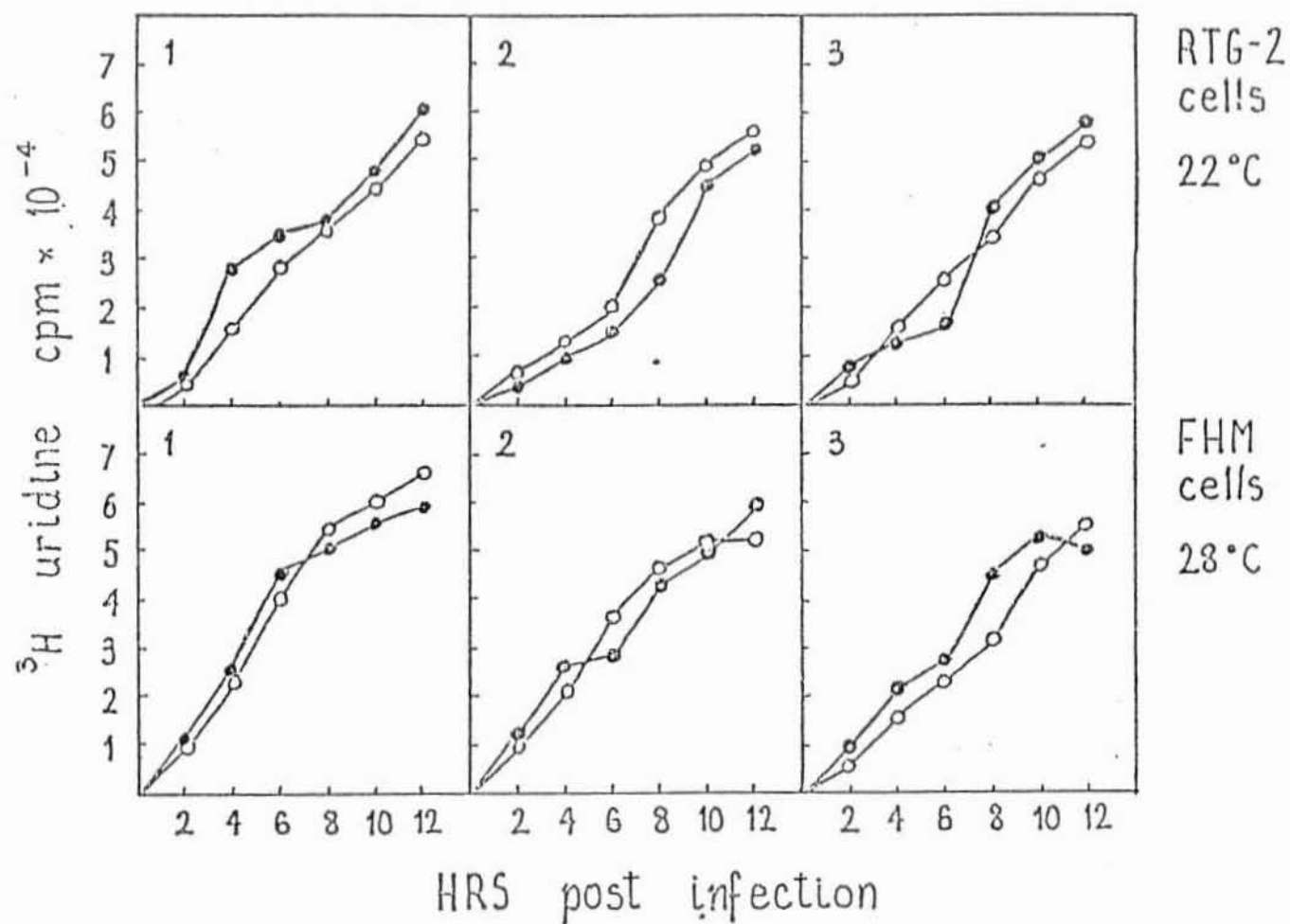
Figure 6. RNA synthesis in RTG-2 and FHM cells. NPV inoculated cells (●) and mock infected cells (○). Days 1, 2 and 3.

DNA Synthesis

The third parameter studied was the effect of NPV on the synthesis of DNA in non-target cells. The experiments were carried out using ^{3}H-Thymidine, a DNA precursor, after inoculation with NPV. The rate of formation of macromolecular DNA was monitored by the rate of thymidine uptake. At days 1, 2 and 3 after inoculation with NPV there was no difference in the rate of ^{3}H-thymidine incorporation between infected and uninfected cells (Fig. 7, 8 and 9). It was also observed that at 28°C the rate of DNA synthesis in L cells (Fig. 7) was much reduced compared to the rate observed at 37°C. It must be emphasized here that reduced rate of DNA synthesis at 28°C is not due to virus infection since mock infected cells behaved similarly at this temperature.

These cumulative data clearly demonstrated the inability of spruce budworm NPV to alter or inhibit the rate of protein, RNA and DNA synthesis in mammalian, avain or fish cell lines. These non-target cells were maintained at their optimum temperatures as well as the temperature optimum for NPV replication. NPV had no effect on the cellular metabolic processes at either temperature. The conclusion derived from these experiments is that NPV neither replicates nor is partially expressed in non-target vertebrate cells.
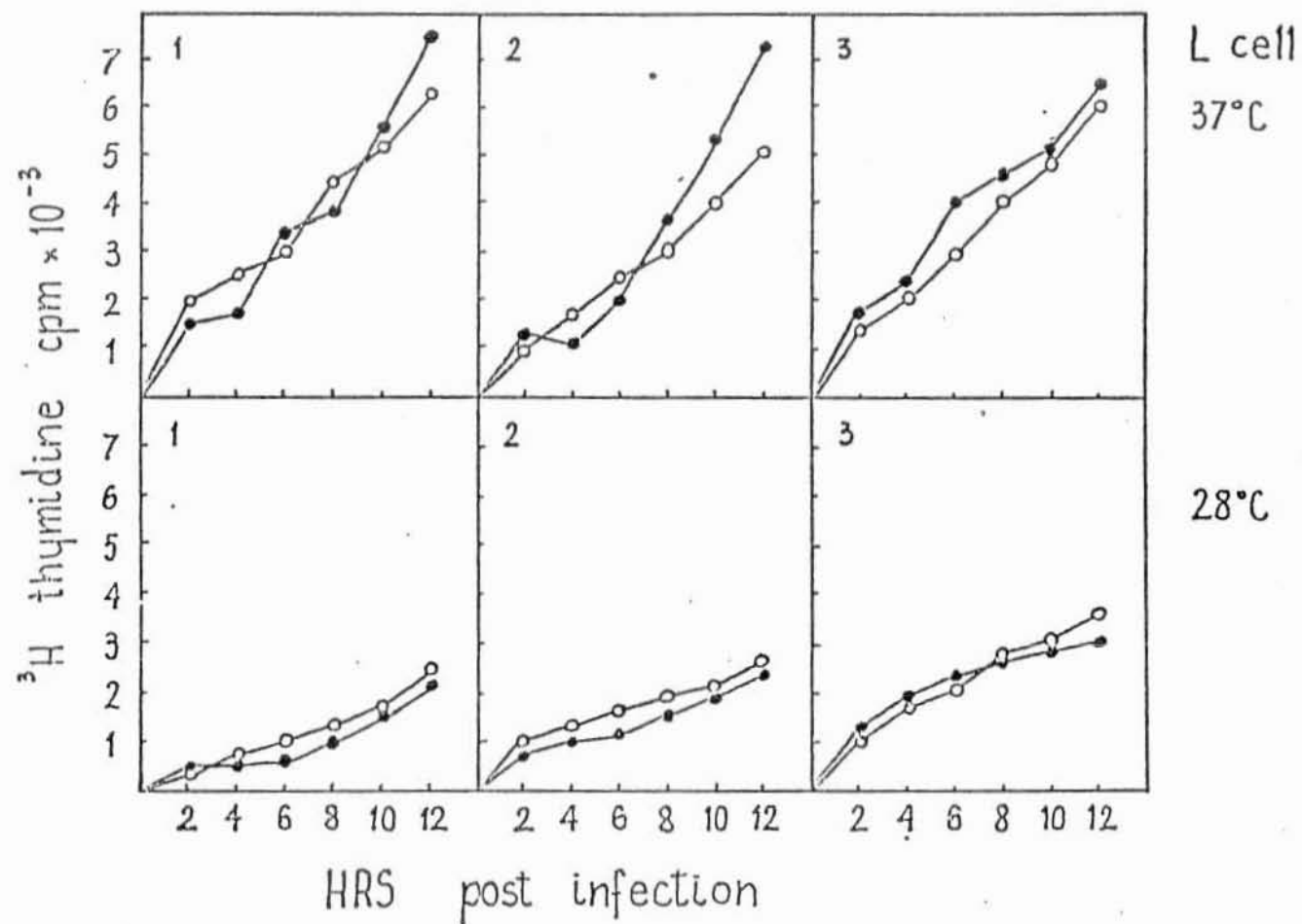
Figure 7. DNA synthesis in L cells incubated at 37°C and 28°C. NPV inoculated cells (●) and mock infected cells (o). Days 1, 2 and 3.
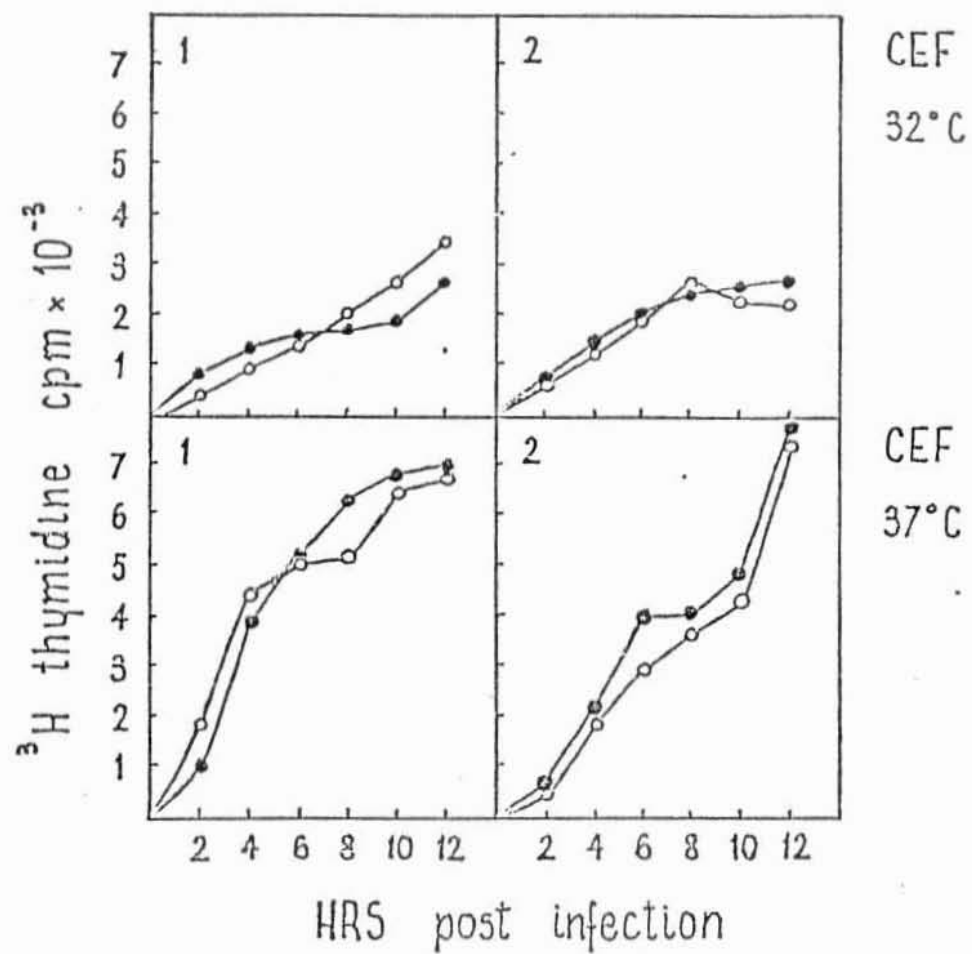
Figure 8. DNA synthesis in CEF cells incubated at 32°C and at 37°C. NPV inoculated (●) and mock infected (o) cells. Days 1 and 2.
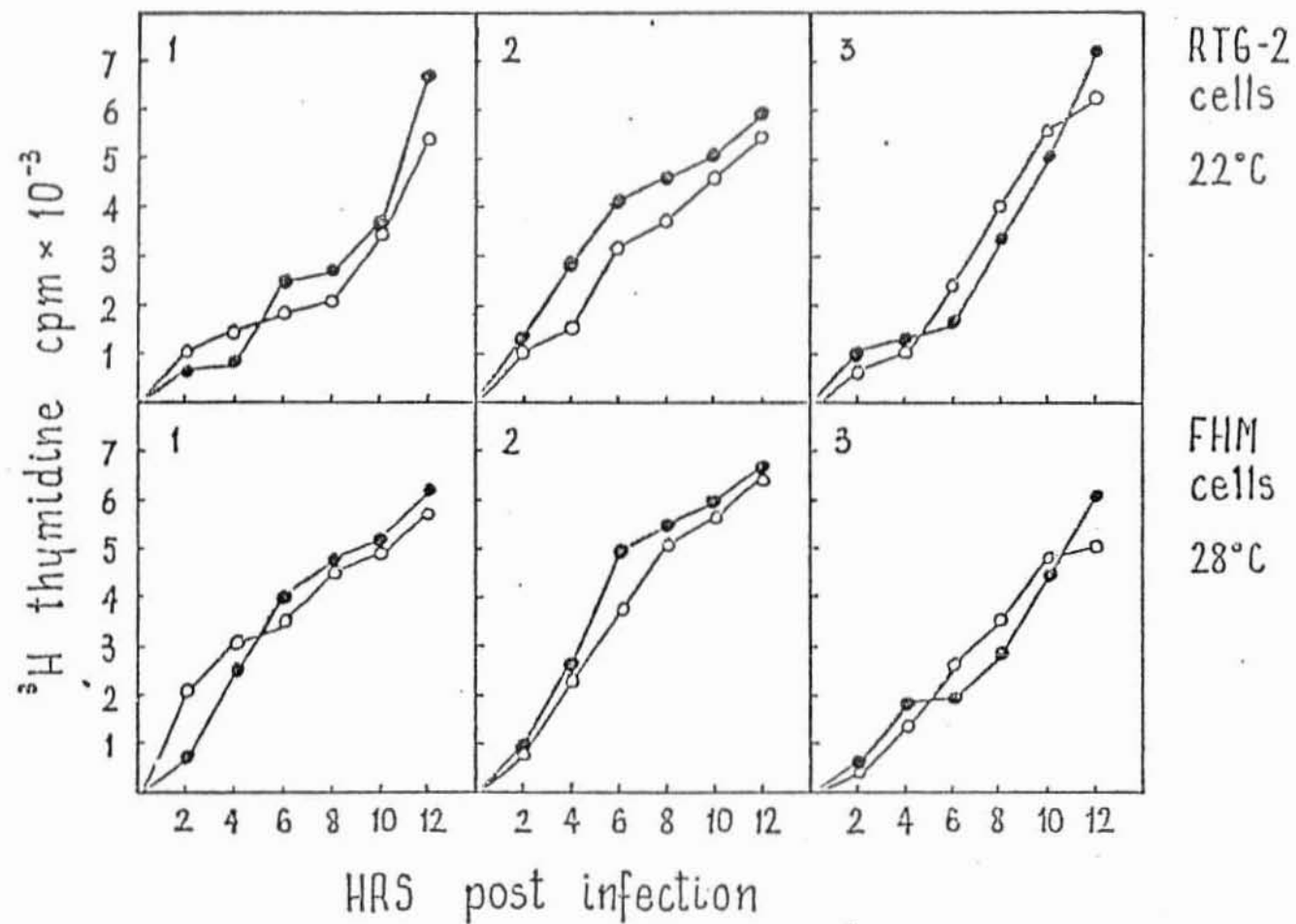
Figure 9. DNA synthesis in RTG-2 and FHM cells. NPV inoculated (●) and mock infected (o) cells. Days 1, 2 and 3.

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