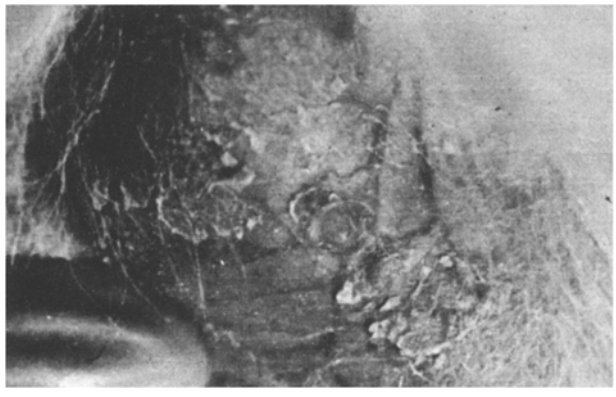
somewhat longer (17–20 μm) with the same curve and characteristic motility as *T. pallidum*. In the serous discharge obtained from the eroded lesion, the number of microbes was about 9×10^7 per ml. The pathological phenomenon in the hares was highly hyperaemic genitals. Some of them had scars and skin lesion. Induration was established neither on the natural infected hare nor on challenged rabbits. The isolation of treponemes from genital lesions of hares and the positivity of their serological test for syphilis speak in favour of a natural treponematosis of the hare.



Lesion developed on the scrotum of a rabbit, after a 110-day period of incubation. Challenge was carried out with the suspension of treponemes isolated from the genitals of a hare *(Lepus sp.)*.

In no far as hunters have never been infected, and treponematosis has never been described on the hands of hunters, it is questionable whether this treponeme is pathogenic for the human. As the serum of hare immobilized treponemes in Nelson (TPI) test an antigenic relationship may exist between *T. pallidum* and treponemes of hares. We must consider the long incubation period after experimental infection of the rabbits and the lack of sexual intercourse between rabbits and hares. It may be presumed that this treponeme detected in the hare is not identical with *Treponema coniculi*, or it may be a variant adapted to the hare. If the future examination gives proof that the isolated treponeme is a new species, the nomination *Treponema leporis* could be suggested, because it was isolated from *Lepus sp.*

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On the inhibitory effect of 2-amino-4,6-dichloropyrimidine on growth of Vaccinia virus[1]

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Summary. 2-Amino-4,6-dichloropyrimidine prevents maturation of Vaccinia virus. Proteins synthesized in the presence of the drug are not assembled into virions.

Several dichlorinated pyrimidines have been found to be endowed with a suppressive effect on the growth of Polio I, Coxsackie B_1, Vaccinia and Herpes simplex 1 virus, while being inactive on Newcastle disease, Vesicular stomatitis and Encephalomyocarditis virus[2]. It has also been demonstrated that the inhibitory effect of dichloropyrimidines on Polio virus is due to irreversible impairment of the ability of structural virus proteins to assemble complete particles[3]. Preliminary data emerging from ongoing research indicate that the growth of Vaccinia virus is inhibited by a similar mechanism.

Material and methods. 2-Amino-4,6-dichloropyrimidine (ADCP) was furnished by Fluka, Nonidet P40 by BDH, ^{3}H thymidine (21 Ci/mmole) and ^{14}C leucine (280 mCi/mmole) by Amersham. 5-Fluoro-2′-deoxyuridine (FUdR, by Fluka) and Cycloheximide (Calbiochem) were used as reference inhibitors of DNA and protein synthesis, respectively. Vaccinia virus was kindly provided by NIH; human aneuploid HEp 2 cells were furnished by ATCC (Rockville). Experiments were carried out on 16-h-old cell monolayers (2×10^6 or 3×10^7 cells per sample, according to the experiment), which were infected with 20 infectious units (IU) per cell at 20 °C for 1 h. Cells were then washed 3 times with Hank's balanced saline solution (BSS, pH 7.3) and incubated at 37 °C in Eagle's minimum essential medium (MEM) supplemented with 2% calf serum (pH 7.3). ADCP was added to the medium at 100 μg/ml, corresponding to $\frac{2}{3}$ of the maximum non-cytotoxic dose (MNCTD)[2].

Infectious virus yield was determined by plaque assay[4], starting from whole cultures (2×10^6 cells/sample) which were frozen and thawed 3 times (−70 °C and +20 °C) and freed of cell debris at 3000 rpm for 5 min. In order to obtain primary virus plaques easily detectable at low magnification (×50), infected subcultures were incubated in liquid Eagle's MEM for 30 h at 37 °C before addition of the same medium solidified with 1% agar (Noble, Difco). Plaques were counted 16 h later. This method was found to have an error of about ±30%.

Synthesis of virus DNA and proteins, as well as their incorporation into complete particles, were followed in cells (3×10^7/sample) infected and incubated as above, which were labelled with ^{3}H thymidine (2 μCi/ml) and ^{14}C leucine (0.5 μCi/ml). Cells were detached from the glass by 0.25% trypsin (Difco 1:250) in Hank's BSS, suspended in the same buffer containing 2% calf serum and centrifuged at 1000 rpm for 5 min. Pellets were then resuspended in 0.5 ml of sodium phosphate buffer (1 mmole, pH 7.4) containing 0.75% Nonidet P40 and incubated in an ice-bath for 15 min, with 3 intermittent strokes (5 sec each) in Vortex. Nuclei were then pelletted at 1000 rpm for 5 min and discarded. 0.05 ml of the cytoplasmic extracts thus obtained, precipitated with 10% trichloroacetic acid (TCA) at 4 °C for 10 min and redissolved in Toluene (0.5 ml,

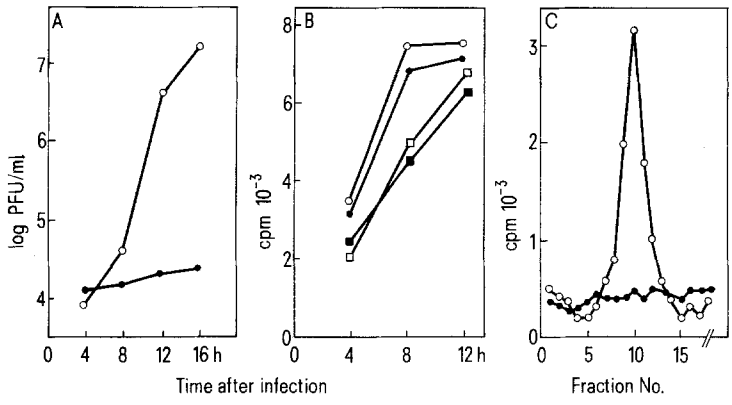

Fig. 1. The effect of ADCP on Vaccinia virus growth. Infected cells were incubated in Eagle's MEM at 37 °C both in the absence (empty symbols) and in the presence (full symbols) of ADCP 100 µg/ml. *A* Infectious virus yield, in plaque forming units (PFU); *B* cell uptake of ^{3}H thymidine (circles) and ^{14}C leucine (squares), added to the cultures at 2 µCi/ml and 0.5 µCi/ml, respectively (2 h pulses), as determined in cytoplasmic extracts; *C* incorporation of ^{3}H thymidine (2 µCi/ml, starting from 2 h post infection) in virionic DNA, determined by density gradient analysis of cytoplasmic extracts 16 h post infection (see methods).

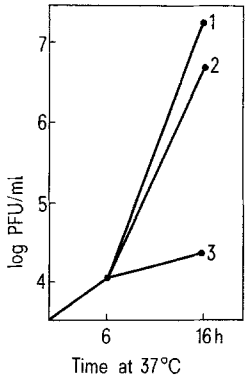

Fig. 2. Vaccinia virus growth (in PFU) in cells incubated for 6 h post infection in Eagle's MEM and then *1* in the same, drug-free medium, or *2* in the presence of FUdR 10 µg/ml or *3* ADCP 100 µg/ml.

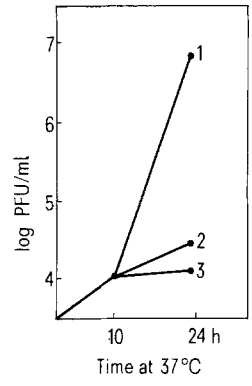

Fig. 3. Vaccinia virus growth (in PFU) in cells incubated for 10 h post infection in Eagle's MEM containing ADCP 100 µg/ml and then *1* in drug-free medium or *2* in the presence of ADCP 100 µg/ml or *3* cycloheximide 20 µg/ml.

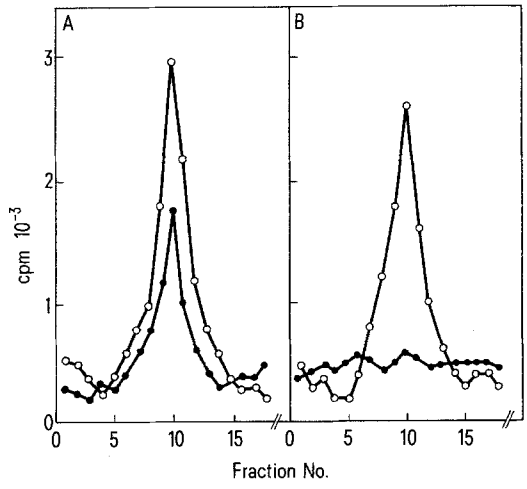

Fig. 4. Irreversible impairment produced by ADCP on the ability of structural Vaccinia virus proteins to assemble complete particles. Infected cells were incubated at 37 °C in Eagle's MEM, both in the absence and in the presence of ADCP 100 µg/ml, doubly labelled with ^{3}H thymidine (2 µCi/ml) and ^{14}C leucine (0.5 µCi/ml) starting from 2 h post infection, and chased with 50 µg/ml of unlabelled thymidine 4 h later. At 10 h post infection, cells were washed in Hank's BSS and incubated in unlabelled drugfree Eagle's MEM for further 14 h. Cytoplasmic extracts were obtained and analyzed by density gradient (see methods). The figure shows the incorporation into complete virus particles of ^{3}H thymidine labelled DNA (empty symbols) and ^{14}C leucine labelled proteins (full symbols) previously synthesized in the absence (A) or in the presence (B) of ADCP.

Packard) was used to determine virus DNA and protein synthesis. 0.3 ml of the same extracts was layered onto 11 ml of sucrose gradient (20–40%) in sodium phosphate buffer containing 0.75% Nonidet P40, spun at 15,000 rpm for 50 min at 4 °C in Spinco (Ti SW 40 rotor) and divided into 40 fractions in order to localize virus peak. Radioactivity was measured by spectrometer (Packard, scintillation liquid: Toluene 666 ml, Triton X 100 333 ml, PPO 7 g, dimethyl POPOP 0.1 g).

Results. As shown in figure 1, ADCP inhibits Vaccinia virus growth without impairing, at least quantitatively, the synthesis of virus (cytoplasmic) DNA and proteins, which, late in the replicative cycle, are mostly structural virionic proteins[5]. The apparent target of ADCP is thus virus assembly, which is strongly reduced. Several facts indicate that, as already observed for Polio virus[3], structural proteins are deeply involved in ADCP antiviral effect. 1. ADCP is still inhibitory on virus growth, even if added 6 h post infection, when as shown by the scarce effect of parallel FUdR treatment virus DNA has already been synthesized while most of the structural proteins are still to be produced (figure 2). 2. If ADCP is removed from infected cells after a 10-h treatment, noteworthy amounts of infectious virus are produced, but on the condition that new proteins are synthesized (figure 3). (Cycloheximide prevents virus 'rescue'). 3. Of virus macromolecules synthesized within 10 h post infection under ADCP treatment, most virus DNA, but very few of the structural proteins, become part of complete particles subsequently produced in drug-free medium (figure 4). Considered together, these data lead us to hypothesize that ADCP (and, conceivably, the other virus-active dichloropyrimidines) inhibits growth of Vaccinia and Polio virus by a common mechanism, i.e. by irreversibly impairing the ability of structural proteins to assemble mature virus particles.

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