probably by checking a thermal-sensitive event in the development cycle.

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Received February 6; revised March 13, 1967.

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Action of Ethidium Bromide on Growth of Herpes Virus in Cell Cultures

ETHIDIUM bromide (EB) (2,7 di-amino-phenyl phenanthridine 10-ethyl bromhydrate) is an antimicrobial drug which, like proflavine (an acridine), forms reversible complexes with nucleic acids[1,2]. *In vitro*, it inhibits DNA-polymerase and, at a lesser rate, DNA-dependent RNA-polymerase[2]. In the same way, EB and related phenanthridine derivatives inhibit the synthesis of nucleic acids in bacteria[3] and Ehrlich ascites cells[4]; they are also trypanocidal[5]. Moreover, the photosensitization of arboviruses[6], the inhibition of their development[7] and that of a *Pseudomonas pyocyanea* phage[8] by these compounds have been reported. Although the responses of several virus-infected systems to the action of acridines have been given in detail[9,10], the comparable interactions with EB have not yet been so clearly described. The present paper details the action of EB on herpes virus and its growth in baboon kidney cells.

We studied first the direct action of EB at 37° C on herpes virus in the absence and presence of light. Rabbit kidney cells, cultured in casein hydrolysate medium with 5 per cent calf serum, were inoculated with herpes virus (strain *IP* 593055). 42 h after inoculation the infected cells were frozen and thawed in their medium and cell fragments were eliminated by centrifugation at 1,000*g* for 10 min. 1 ml. of the virus suspension was distributed into each of 13 flat 60 ml. flasks. 0·5 ml. culture medium

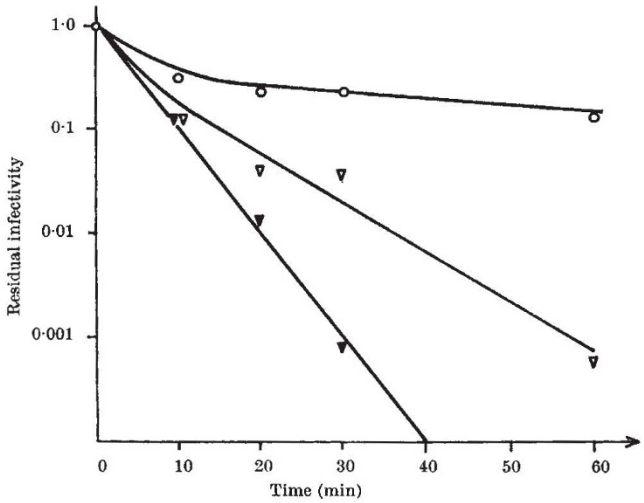

Fig. 1. Action at 37° C of EB on herpes virus in the presence and absence of light. ○—○ μg EB/ml.; ×—×, 1,000 μg EB/ml.; ▼—▼, 1,000 μg EB/ml. + light.

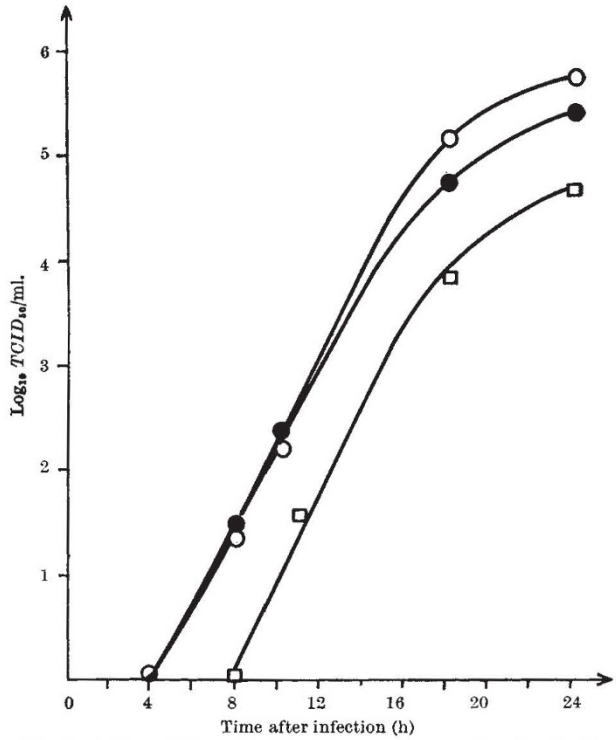

Fig. 2. Action of EB on kinetics of herpes virus growth. ○—○, Untreated controls; ●—●, 1,000 μg EB/ml. 2 h before virus inoculation; □—□, 1,000 μg EB/ml. from the second to the fourth hour after virus inoculation.

containing 3 mg EB/ml. was added to eight of the flasks, and half of these were wrapped in aluminium foil to keep out the light. The five remaining flasks received 0·5 ml. of medium with no EB. All flasks were then kept for various periods at 37° C, 15 cm from three cold white lamps with a total power of 100 W. The residual virulence of each sample was immediately determined on baboon kidney cells by inoculating ten-fold dilutions, each dilution being inoculated into five tubes. The cytopathogenic effect was read on the third day after inoculation, and titres calculated according to the method of Reed and Muench[12] and expressed as $\log_{10}$ $TCID_{50}$/ml. Fig. 1 shows that EB has both an inactivating and a photosensitizing activity with herpes virus.

Because of this direct effect, the infected cell system should be exposed to EB only during the eclipse phase in order to study the effect of EB on herpes virus growth. 60 ml. flasks containing baboon kidney cell cultures in Eagle's medium[13] with 10 per cent foal serum were distributed into three batches, two of which were inoculated with herpes virus, the multiplicity of infection being approximately 30. After incubation at 37° C for 1 h, the inoculum was removed and the cell layers washed five times with Hanks fluid. Non-absorbed virus was then neutralized for 1 h at 37° C with a hyperimmune rabbit serum, which was afterwards removed. After three washings, each culture was placed for 2 h at 37° C in contact with 2 ml. of a solution of 1 mg/ml. EB in an Eagle medium containing 5 per cent foal serum for the first batch of tubes and 2 ml. of medium for the second batch. The supernatants were removed, the cells washed five times and 5 ml. Eagle medium containing 5 per cent foal serum added to the cell cultures. The cultures were incubated at 37° C. Flasks containing the third batch were incubated with EB for 2 h before inoculation. At different times, two cultures of each batch were frozen and thawed three times, mixed, and titrated.

Fig. 2 shows that EB has little effect on the kinetics of viral growth when added 2 h before inoculation at the maximal concentration which does not modify the cell

viability for 24 h. On the other hand, when EB is added from the second to the fourth hour after inoculation, there is a delay of 4 h before viral growth begins, although growth from then on is normal.

The photosensitivity of virus grown on cells treated with various stains has been reported for certain arboviruses[6] and for polio virus[14]. In our experiments baboon kidney cell cultures were inoculated with herpes virus, and half of them were then treated with EB from the second to the fourth hour following inoculation. All the cultures were collected 24 h after inoculation, and submitted to three cycles of freezing and thawing. Viral suspensions obtained after centrifugation were illuminated at 37° C for 2 and 4 h under the conditions described. The residual infectivity of the virus from treated cells was 1·6 per cent after 2 h and 0·1 per cent after 4 h of light treatment; it was 7·6 and 1·6 per cent for virus grown in the cells not treated with EB. This finding shows that the virions formed by cells treated with EB also have a certain photosensitivity.

The direct action of EB on normal herpes virus as well as the properties of virus grown in cells treated with EB may be attributed to binding of the stain on the viral genome, which could inhibit its expression or favour its degradation under the action of light. The combination of EB with cellular and viral DNA, causing inhibition of cell synthesis (at high concentrations of dye) and blocking the transcription and duplication of viral DNA, probably also accounts for the effect of EB during the virus eclipse phase. The temporary character of this inhibition is interesting in view of the known *in vitro* reversibility of the DNA–EB complex[1].

We thank Dr Karl Habel and Dr Gérard Orth for helping us write this manuscript and for very fruitful discussion. We also thank Miss Marie-Louise Ryhiner for excellent technical assistance. This work was aided by the Institut National de la Santé et de la Recherche Médicale.

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Received February 20, 1967.

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Isolation of SV_{40} Virus and its Effect on Renal Function in African Green Monkeys

SV_{40} virus is of considerable concern, because it has been shown to be present in a number of samples of inactivated polio virus and adenovirus vaccines produced before 1960, and it is capable of inducing both chromosomal changes and tumours in certain experimental systems. Since the isolation of SV_{40} virus from naturally infected monkeys was reported by Sweet and Hilleman[1], several investigators have examined experimental infection in monkeys and man with this agent. It has been shown that infection can be produced in African green monkeys by several routes of inoculation[2,3]. Inoculation of human volunteers[4–7] has resulted in low grade infection including some evidence of transient chromosomal changes. Furthermore, administration of this virus to hamsters has resulted in the production of tumours[6,8].

The experiments reported here were designed to investigate the effects of intravenous infection of the African green monkey with SV_{40} virus and to investigate the chief sites of viral growth and the possible effects on renal function in this primate.

African green monkeys (*Cercopithecus aethiops tantalus*) were used. When these animals arrived, they were first quarantined for 3 weeks and then transferred to individual sterile cages. They were tuberculin tested and checked for other infections. The animals were pre-tested for the presence of antibodies to SV_{40} virus before infection. Only animals free of antibodies were used. SV_{40} virus adapted to African green monkey kidney culture was inoculated intravenously using 1 ml. of infected tissue culture fluid which contained 5·2 $TCID_{50}$ ($\log_{10}$) of virus (experimental animals) or 1 ml. of control infected tissue culture fluid (control animals). The animals were divided into three groups, and each group consisted of two experimental monkeys and one control. Group 1 animals were killed 2 days after inoculation; group 2 animals were killed 2 weeks after inoculation; and group 3 animals were killed 2 months after inoculation.

Blood and urine specimens were obtained at 2–5 day intervals for 4 weeks, and at weekly intervals for an additional 4 weeks. Kidneys (separated into medulla and cortex), lymph nodes, spleen, liver and lung from dead animals were tested for virus and examined for gross and microscopic pathological changes.

Virus infectivity titres and neutralization tests on all virus isolates from every tissue tested were determined in primary tissue culture cells of African green monkey kidney (AGMK) free of SV_{40} virus. Inoculated AGMK tube cultures were incubated in the stationary phase at 37° C and examined microscopically from days 8 to 14 after inoculation for cytopathic effect. Appropriate controls were maintained in each test. Infectivity and neutralization 50 per cent end points were calculated by the method of Reed and Muench[9].

Laboratory tests[10] to detect possible renal damage and other functional changes were conducted on all surviving animals at 2 or 5 day intervals for 4 weeks, and at weekly intervals for an additional 4 weeks. The tests utilizing the blood specimens included sedimentation rate, total serum protein, albumin/globulin ratio, blood urea nitrogen and cholesterol, Uncentrifuged urine specimens were tested for specific gravity, *p*H, albumin, sugar, acetone, red blood cells, white blood cells, and casts (also red and white cells and casts after centrifugation).

The concentrations of infectious virus recovered from organs of monkeys killed 2 days, 16 days and 60 days respectively after intravenous inoculation of SV_{40} are shown in Table 1. The titres of virus in infected animals 2 days after infection were larger in the cortex of the kidney, the lymph node, spleen and lung. Sixteen days after infection concentrations of virus in these organs had increased by factors of 2–3 $\log_{10}$ with the greatest increase in the kidney medulla (3·5 log increase). The concentration of virus in the lung, however, decreased ten-fold. These results suggest that virus multiplication is limited to the spleen and kidney in particular and the reticulo-endothelial system in general. Although virus was recovered from all organs tested 2 months after infection, the concentrations were less than ten infectious particles/ml. of 10 per cent suspension of tissue homogenate.

Fig. 1 shows the concentrations of SV_{40} virus recovered from the urine and blood at different times from 2–60 days after infection. Viraemia was detectable within 2