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Influence of Actinomycin D, Aurantine and 5-Bromdeoxyuridine on Production of Nucleic Acid and Protein in Measles Virus. (30602)

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Previous experiments have established that viral RNA itself is the matrix for viral RNA synthesis and not the cellular DNA. Substantiating these conclusions are numerous experiments with actinomycin D, an antibiotic which forms a complex with the DNA molecule(1) thereby inhibiting the activity of RNA-polymerase and of the cell RNA synthesis; to a lesser extent this is also true for the DNA-polymerase and DNA synthesis (2,3). Experiments with actinomycin D have shown that the reproduction of RNA-containing viruses—Mengo(4), Newcastle Disease (5), phage MSE(6), encephalomyocarditis of mice(7), Sendai(8) and Venezuelan equine encephalitis(9)—is not dependent on the cell DNA whereas the protein and nucleic acid synthesis of RNA-containing influenza virus depended upon the cell DNA(5,8).

That DNA does not participate in the synthesis of the majority of RNA-containing viruses is also confirmed by the fact that 5-bromdeoxyuridine and other thymine analogs which inhibit the synthesis of the cellular DNA and viral DNA do not inhibit reproduction of the RNA-containing viruses, such as poliomyelitis(10,11), vesicular stomatitis (10), arboviruses(11,12,13), Newcastle Disease(13) and others. Inhibition by 5-fluordeoxyuridine and by actinomycin D of the RNA-containing Rous sarcoma virus is explained by the influence of the viral RNA

TABLE I. Effect of Actinomycin D upon Measles Virus Multiplication (in Continuous Human Amnion Cell Culture).

Days	Measles virus	Measles virus + actinomycin	Days	Measles virus	Measles virus + actinomycin
1	—	—	7	5.0	5.0
2	—	—	8	—	—
3	1.0*	1.0	9	5.0	5.0
4	—	—	10	—	5.0
5	4.0	5.0	11	4.0	1.5
6	5.25	5.25	12	—	—

* Log TCD_{50}.

upon the cell genome and by appearance in the cell DNA of complementary properties for the viral RNA.

The purpose of the present study was to investigate some aspects of measles virus synthesis. The nucleic acid of this virus has not been isolated nor has its type been determined by direct biochemical methods. However, according to electron microscopic studies, the structure of measles virus has been shown to be similar to that of the RNA-containing myxoviruses(14) and, furthermore, inhibitors of DNA synthesis do not prevent reproduction of measles virus(10,12) nor of other RNA-containing viruses.

Materials and methods. Two strains of measles virus (L-4 and Ledwin), were propagated in trypsinized chick embryo fibroblasts, human amnion cells or HeLa cells. Measles virus in the amount of 10^4 to 10^5 TCD_{50} in 0.1 ml of culture fluid was added to each tube containing 1.0 ml of Medium 199 with 5% bovine serum. Actinomycin D (0.1 μg/ml) was added after infection to the human amnion cell culture (Table I); aurantine (1.0 μg/ml) to the chick embryo fibroblast culture (Table II), and 5-bromdeoxyuridine (15 μg/ml) to the HeLa cell culture (Table III). Aurantine is a compound similar in structure to actinomycin C which was generously provided for these studies by V. S. Soloviova. The course of infection in the culture fluids was assayed by cytopathic effects. Hemagglutinating activity was determined with 0.5-1.0% suspension of goose erythrocytes at room temperature.

TABLE II. Effect of Aurantine upon Measles Virus Multiplication (in Primary Trypsinized Chick Embryo Fibroblast Culture).

Days	Measles virus	Measles virus + aurantine	Days	Measles virus	Measles virus + aurantine
1	—	—	7	3.0	3.0
2	—	—	8	—	—
3	—	—	9	1.75	1.0
4	1.0*	1.4	10	2.0	2.0
5	2.0	—	11	4.0	3.29
6	4.0	4.0	12	2.0	1.5

* Log TCD_{50}.

Results. The presence in the medium of actinomycin D (Fig. 1), aurantine (Fig. 2) or of 5-bromdeoxyuridine (Fig. 3), had practically no effect upon the replication of measles virus. Despite the low hemagglutinating activity of measles virus, the present study showed that the accumulation of hemagglutinin was similar both in the presence and in the absence of actinomycin in the medium.

TABLE III. Effect of 5-Bromdeoxyuridine upon Measles Virus Multiplication (in HeLa Cell Culture).

Days	Measles virus	Measles virus + 5-bromdeoxyuridine
1	—	—
2	2.0 *	2.36
3	2.63	2.63
4	—	—
5	—	2.60
6	4.6	4.29
7	—	3.25
8	3.41	3.25
9	2.75	—
10	2.0	3.5
11	—	—
12	—	—

* Log TCD_{50}.

When tissue cultures infected with measles virus were stained with acridine orange, lesions typical of measles infection(15) were observed: giant multinuclear cells; regrouping and accumulation of green fluorescent material in the nucleus, particularly around the nucleolus, and in the perinuclear zone of the cytoplasm; poor orange staining of the cytoplasm. Cytopathic effect was marked. When noninfected tissue cultures were treated with actinomycin or aurantine the following cellular changes were observed: regrouping of the DNA material of the nuclei; shrinking and disintegration of the nucleoli; disruption of

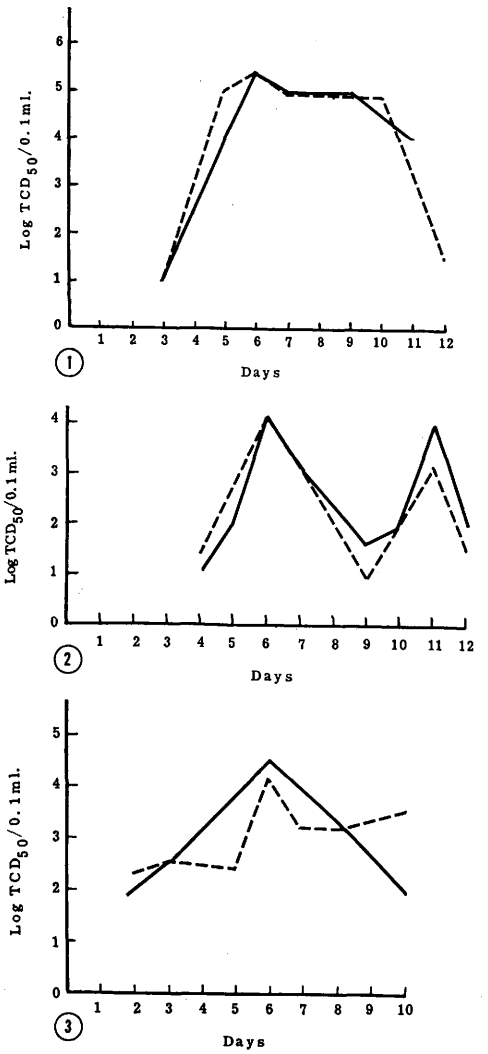

FIG. 1. Effect of actinomycin D (0.1 μg/ml) upon measles virus multiplication in continuous human amnion cell culture.

FIG. 2. Effect of aurantine (1.0 μg/ml) upon measles virus multiplication in trypsinized chick embryo fibroblast culture.

FIG. 3. Effect of 5-brom-2-deoxyuridine (15 μg/ml) upon measles virus multiplication in trypsinized chick embryo fibroblast culture.

the DNA material associated with the nucleolus; disappearance of nucleoli; vacuolation of the nucleus; poor orange staining of the nucleoli and of the cytoplasm. In the presence of actinomycin D or aurantine growth of cells was delayed, as compared to control cultures. The cellular changes observed in uninfected cultures in presence of actinomycin were also seen in the cells infected with measles virus treated with actinomycin D or aurantine, but the cytopathic effect of measles virus was superimposed on these changes thereby accentuating the cytopathic effect.

The evidence obtained by propagation of measles virus in the presence of actinomycin D and aurantine indicates that the synthesis of the nucleic acid and protein of measles virus occurs without participation of the cell DNA as the matrix. The lack of effect of 5-bromdeoxyuridine upon the development of measles virus is additional confirmation that DNA takes no part in the synthesis of this virus. From these observations we may indirectly adduce that measles virus contains RNA.

Summary. The propagation of measles virus in tissue cultures was not affected by actinomycin D, aurantine, or 5-bromdeoxyuridine. From these findings it is adduced that measles virus contains RNA.

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A Therapeutic Antiviral from an Extract of λ Infected *E. coli* (Phagicin).* (30603)

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Inhibition of viral cytopathogenicity and viral replication by materials derived from bacteria has been reported(1,2). The most recent reports have dealt primarily with the presence of cytopathic effects in cultures treated with lysates of some bacteria such as *Corynebacterium* and *E. coli* K-12(3,4). Since the bacteria involved are known to contain prophages in their genome, it seemed important to determine whether bacteriophage might be responsible for the antiviral activity against animal viruses.

The *E. Coli* K-12 was chosen as a model system because its metabolism and genetic structure are well known and it harbors a temperate phage lambda which has been intensively studied. In this system it is possible to document phage-specific immunity, interference and phage conversion. Although there is to date no evidence of interference between bacteriophages and animal viruses it seemed to us worthwhile to study the possibile activity of *E. coli* lysates containing bacteriophage against herpes and vaccinia viruses.

Methods and materials. Bacterial strains. The strains of *E. coli* K-12 (λ) and *E. coli* λ sensitive used in this investigation were obtained from Dr. W. Dempsey, Biochemistry Dept., University of Florida.

Media. Media used for propagation of bacterial strains was Difco Pen Assay Broth. Same media containing 0.8% agar, 0.5% NaCl and 10^{-3} M Mg $SO_4 \cdot 7H_2O$ was used for the agar overlay technique.

Preparation of phage lysates. A stock of phage was obtained by U.V. induction of an exponentially growing culture of *E. coli* K-12 (λ). The lysate was filtered, stored at 4°C and subsequently used as an inoculum to infect *E. coli* λ sensitive for production of high titer lambda phage lysates. The phage stock used throughout this work was prepared by the agar overlay technique(5). Young cultures of a lambda sensitive strain of *E. coli* were suspended in distilled water containing 10^{-3} M Mg $SO_4 \cdot 7H_2O$ and 0.5% NaCl, to a concentration of 10^7 cells per ml. One ml of the bacterial suspension was infected with λ phage, placed in 9 ml of soft agar, allowed to absorb for one hour at 46°C and poured over a hard trypticase soy agar base in 32 oz bottles. The bottles were incubated at 36°C for 18 hours at which time the soft agar layer was removed, broken and suspended in Pen Assay Broth. The soft agar and bacterial debris were removed by cenrifugation and the supernatant fluid was filtered through millipore filters in the cold. A final filtration was done through a sterile 0.45 μ millipore, and this lysate was used for antiviral activity assays both *in vivo* and *in vitro*. The phage titer of each lysate was determined by counting the number of plaques produced by a diluted sample of the phage lysate on a layer of sensitive indicator strain.

For biochemical determinations the plaques were washed with lambda diluent(6) consisting of 1 M Tris buffer pH 7.5; 0.12 g of Mg SO_4, $7H_2O$; 4 g NaCl and 0.05 g gelatin per liter. In nucleic acid analyses, an aliquot of the lysate was incubated with dilute alkali at 37°C for 18 hours followed by acidification with trichloroacetic acid. The RNA was measured in the acid soluble supernatant with the orcinol reaction(7). The DNA was ex-

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