A Sensitive Assay for Infectivity of Mengovirus RNA In L Cells. (32074)

FERDINANDO DIANZINI,* SAMUEL BARON, AND CHARLES E. BUCKLER
U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Biology of Viruses, Bethesda, Md.

A technique for assay of infectious polio RNA which shows a higher sensitivity than the standard techniques previously used has recently been described(1). This procedure has been carried out using HeLa cells suspended in isotonic medium as "absorbing cells" and a more concentrated HeLa cells-agar suspension as "indicator cells." The "absorbing cells" suspensions were exposed to polybasic substances plus dilutions of infectious RNA and, after a proper period of time, were mixed with the "indicator cells," overlaid on petri dishes containing a preformed layer of nutrient solidified medium, and incubated in a humidified CO_2 incubator at 37°C. In this way, the virus produced by the RNA-infected "absorbing cells" can be transmitted to the contiguous "indicator cells" and thereby produce plaques which become fully evident in 24-48 hours without any staining procedure.

The main advantages of this procedure are: *a*) the use of suspended cells in isotonic medium(2), and *b*) the possibility of using compounds which increase the uptake of infectious RNA by the cells(3-7) and confining toxic side effects of these compounds to the "absorbing cells." The present study was undertaken to determine the best conditions for the assay of Mengovirus RNA in suspended mouse L cells using enhancing compounds.

Materials and methods. L cells (strain CCL-1) were grown in suspension in Eagle's spinner medium supplemented with 10% calf serum. Mengovirus RNA was extracted from infected L cells by the hot phenol method(8). Five μg/ml of polyvinyl sulfate were present in the aqueous phase. The infectivity of this preparation was completely lost after treatment with RNAse (10 μg/ml) at 37°C for 20 minutes. Poly-L-ornithine, mol. weight 45,000 (PO) was obtained from Mann Research Laboratories and from New England Nuclear Corp. Diethylaminoethyl-Dextran (DEAE-D) mol. weight 2×10^6 (Pharmacia) was a gift of Dr. K. Takemoto.

The assay procedure was basically the same as described by Koch *et al*(1) with minor modifications included in the description below: 0.3 ml of cell suspension in PSM† containing 10^8 cells per ml were mixed with 0.2 ml of a given dilution of PO in PSM. After 5 minutes at 37°C the suspension was diluted 10-fold with PSM and 0.3 ml was mixed in a test tube with 0.1 ml of Mengovirus RNA (diluted in PSM containing a given concentration of DEAE-D) and with 0.1 ml of PO solution in PSM. This mixture was allowed to incubate in a water bath at 37°C for 60 more minutes and then 1 ml of the suspension of "indicator cells" in Eagle's spinner medium supplemented with 2% calf serum ($1.5 - 2.0 \times 10^7$ cells per ml) was added to each tube. Previously a series of tubes containing 0.5 ml of Eagle's spinner medium supplemented with 1% calf serum, 1.4% of agar (Noble Agar Difco) and 0.015% of DEAE-D‡ (kept melted at 56°C) were prepared. Also prepared were a series of plastic petri dishes (60×15 mm) containing 5 ml of the same medium supplemented with 3% calf serum.

Immediately after the "indicator cells" were mixed with the "absorbing cells", samples of 0.5 ml were added to the tubes containing the melted agar medium and the entire contents were overlayed on the prepared petri dishes. Plaques were counted after 24-48 hours of in-

* Institute of Microbiology, University of Siena, Italy. Presently a guest worker at NIH under a fellowship from Italian National Research Council, Group of Experimental Medicine.

† 0.15 M NaCl; 0.02 M sodium phosphate buffer; 0.001 M $MgCl_2$.

‡ Included in the medium in order to inactivate the agar-inhibitors for Mengovirus(9).

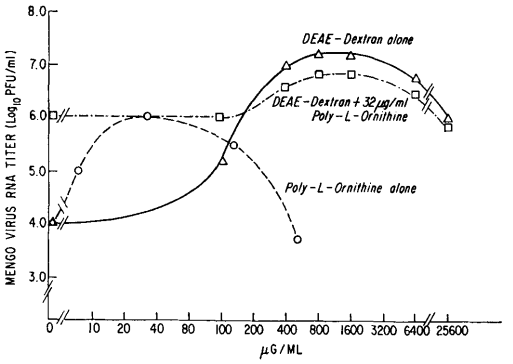

FIG. 1. Effect of various concentrations of poly-L-ornithine and DEAE-dextran on infectious titer of mengovirus RNA. For the curve in which the enchancing compounds are combined poly-L-ornithine was held constant at 32 μg/ml and DEAE-dextran was varied.

cubation in humidified 4% CO_2 environment at 37°C. Two plates were used per each dilution and the experiments were repeated several times.

Results and discussion. The curves plotted in Fig. 1 show the effect of varying concentration of DEAE-D and PO on the titer of Mengovirus RNA either when these compounds were used alone or in combination. When neither DEAE-D nor PO were used, the titer was $10^{4.0}$ PFU per ml. PO caused a 2 $\log_{10}$ enhancement of the titer when used at the optimal concentration of 32 μg per ml. Lower doses were less efficient; higher doses caused a fall of the yield perhaps because of toxicity on the "absorbing cells." DEAE-D shows a similar behaviour but at the optimal concentration, between 800 and 1600 μg per ml, the increase of titer was more than 3 $\log_{10}$. The changes of infectivity which occurred during combined treatment with the optimal dose of PO (32 μg per ml) and variable concentrations of DEAE-D, reflect the effect of each compound when used separately. When low and relatively ineffective concentrations of DEAE-D were used, the increase of titer was apparently determined by the more effective PO. Analogously, with more efficient concentrations of DEAE-D, the yield did not exceed that produced when the same concentrations of this drug were used alone. Any additive effect of PO to DEAE-D would not measurably change the final yield of virus. These findings indicate that DEAE-D and PO act independently and without any evident synergistic effect, thereby confirming the observations by Koch *et al*(1) in the HeLa cells-poliovirus RNA system. However, although PO was more active than DEAE-D in the HeLa-polio RNA system, DEAE-D was more effective than PO in the L cells-Mengovirus RNA system. Application of the same procedures to cell monolayers rather than to suspended cells gave the same overall results except that sensitivity to infection was 10-fold lower than in suspended cell cultures.

The present findings do not help to determine whether DEAE-D and PO increase infectivity by stimulating cellular uptake of RNA or by protecting RNA from the action of nuclease(1,3,5,10). The results do confirm the relatively high sensitivity to viral RNA of suspended cells treated with polybasic substance(1). However, the findings indicate that optimal conditions for infection by viral RNA can vary widely for different RNA-cell systems.

Summary. A sensitive method of assay for Mengovirus RNA has been investigated using an agar-L cells suspension plaque technique as previously described for poliovirus RNA-HeLa cells system. The activity of polybasic substances (DEAE-dextran and Poly-L-Ornithine) at different concentrations also has been studied. Both compounds cause an increase of infectious titer of Mengovirus RNA at a rate depending on their respective concentrations. When used at the optimal concentrations, DEAE-D shows enhancing activity of about one thousand times and PO of one hundred times with respect to the control conditions. When the drugs were used in combination, no synergistic effect was evident. The highest infectious titer ($10^{7.2}$ PFU per ml) was obtained using DEAE-D at a concentration of 800-1600 μg per ml as diluent fluid for the infectious RNA preparations.

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Homeothermic Development of the Young Chick.* (32075)

DAVID R. WEKSTEIN AND JAMES F. ZOLMAN (Introduced by P. A. Thornton)
Department of Physiology and Biophysics, College of Medicine, University of Kentucky, Lexington

Newly hatched domestic chicks are poor thermoregulators but rapidly develop thermoregulation(1,2). The functional basis of this ontogenetic development of thermoregulation is not known. The increase in insulation by the growth of down and feathers, and the decrease in the ratio of surface area to body mass, both of which occur during the first week of rapid growth, generally are accepted as important contributions to effective thermoregulation by the young chick. Maturation of neural and hormonal mechanisms, and an increase in rate of thermogenesis are other suggested contributors(3,4,5). In our experiments, homeothermic development of the chick, and the effects of increases in body weight, food intake, and down on this development were studied.

Methods and procedures. Arbor Acre x Vantress chicks, with known hatch times, were used. Some chicks were hatched in our laboratory; each chick was banded and the time of hatch recorded within one hour after emergence from the shell. Other chicks were obtained from a commercial hatchery with a hatch time range of one hour. All chicks were reared in communal cages of a 35°C brooder. Three groups of chicks were used in these experiments: (a) normal chicks reared with food and water available *ad lib* in the brooder; (b) normal chicks reared for 40 hours without food and water; and (c) "shaved" chicks with the down removed from their bodies by the topical application of calcium thioglycolate 5 hours before cold stress. The shaved chicks were reared with food and water available *ad lib*.

In the first experiment, 95 free-feeding normal chicks of 5 hatch times (8-, 16-, 32-, 64-, and 128-hrs. old) were taken from the brooder at different times, weighed, and placed in a lighted incubator set at 35.5°C. One hour later, the chicks were removed quickly from the incubator and housed individually in small boxes (15 × 15 × 15 cm) in a 10°C control temperature room. Cloacal temperatures were recorded immediately from a Yellow Spring Telethermometer connected to a calibrated tissue implantation probe (Yellow Springs Model 511) that was inserted manually 2.5 cm in the cloaca. Every 15 minutes, thereafter, cloacal temperatures were measured during a one-hour exposure period. The probe readings were later converted to temperature values.

In the second experiment, 12 chicks reared without food and water until they were 40 hours old were studied. Sixty-four "shaved" free-feeding chicks of 3 different hatch times were used in the third experiment. Handling and recording procedures were the same for the 3 experiments.

Results and discussion. The cooling curves for the 5 groups of free-feeding normal chicks

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