about 5.5 ± 1.2 times more sensitive than VSV and 1.4 times more sentitive than SFV when the resultant PDD_{50} units were compared.

Discussion. Interferon preparations induced in chick embryo cell cultures by several viruses gave parallel dose response curves allowing estimates to be made of the relative potencies of each interferon preparation(1). When different viruses are employed to assay an interferon preparation the slope of the dose response lines may differ significantly(1). These differences in slope may be due to variations in the assay systems for the various challenge viruses. It is not valid to make a comparison of the sensitivities of two viruses to interferon if the slopes of the dose response lines differ markedly since the differences between the assays will depend upon the definition of the unit of activity which is arbitrarily chosen. In this study a valid comparison could be made since the slopes of the VSV and vaccinia dose response lines were similar. It has been previously shown that the chikungunya assay was 2.9 times more sensitive than the vaccinia assay system as described. The present study extends this observation by indicating that the vaccinia assay is about 5.5 times more sensitive than the VSV assay and 1.4 times more sensitive than the SFV assay system under the conditions imposed. The relative sensitivities of VSV and vaccinia are comparable with that obtained in different laboratories with the same interferon preparation(5).

These comparisons are somewhat different from those reported by Ruiz-Gomez and Isaacs(6) but probably reflect the greater sensitivity of the vaccinia assay used herein whereby the interferon is continuously present during plaque formation.

Summary. Assays of interferon in chick cell cultures were performed using vaccinia, Semliki Forest and vesicular stomatitis viruses as challenge viruses. Vaccinia virus was found to be approximately 5.5 times more sensitive than vesicular stomatitis virus and 1.4 times more sensitive than Semliki Forest virus to the action of interferon under the conditions of the assays.

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Early Effects of SV_{40} on Growth *in vitro* of Hamster and Human Tissue Cells.* (31347)

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To investigate the early events after infection *in vitro* of primary cells by SV_{40}, suspensions of cells from several organs were exposed to the virus and then propagated simultaneously in soft agar and in monolayer tube culture. It was reasoned that by exposing cells to virus under these conditions a more complete representation of the cellular spectrum present in the tissues might be brought into contact with virus, since it is likely that a certain degree of selection occurs when cells are cultivated *in vitro*

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even for a short time, as has been the usual procedure in studying virus-cell interactions. In these experiments the soft agar technique (1) was employed to distinguish any early increase in growth potential of cells exposed to SV_{40}. Cells were also grown at the same time in monolayers to determine whether infection and subsequent transformation of the cells would occur after exposure in suspension.

Materials and methods. Virus. SV_{40}, strain VA 45-54 obtained from Dr. M. R. Hilleman, was propagated in grivet monkey kidney cells, line AH-1(2). Two stock suspensions were employed, the titers of which were respectively $10^{5.2}$ and $10^{6.2}$ $TCID_{50}$/0.1 ml as determined in AH-1 cells.

Cells. Hamster embryo cells were prepared from whole Syrian hamster embryos of an estimated 2-weeks gestation. However, in 2 experiments in which such tissues were employed, the embryos in the first were approximately twice as large as in the second. Embryos were minced and then trypsinized for 4 hours in 0.25% trypsin. During this period the trypsin solutions were twice removed and replaced. The cell suspension then consisted almost entirely of single cells. The cells were washed once and resuspended in 40 ml of Puck's saline A to a final concentration of approximately 3×10^5 cells per ml. Newborn hamster lung cells were prepared from organs of a litter of newborn Syrian hamsters as just described. Lung tissue did not disperse as readily as hamster embryo tissue; even after 4 hours treatment with trypsin a number of small clumps of cells remained. Human kidney cells were obtained from a kidney of a 3-month-old infant. The cell suspension was prepared as described above.

Exposure to SV_{40}. To 20 ml of the 40 ml cell suspension, SV_{40} virus was added to a multiplicity of infection of approximately 1. The cell-virus mixture was kept for 1½ hours at room temperature and slowly agitated by a magnetic stirrer. The remaining 20 ml of suspension was used as control and was maintained under the same conditions. After adsorption, the virus-exposed and control cells were washed twice in Puck's saline A. Half of the cells in each suspension were resuspended in 10 ml of medium and added in aliquots of 1 ml to 16 × 150 mm tubes. The remainder of the suspension was employed in preparing soft agar cultures. During the first week the tubes were incubated in in a stationary position at 37°C. Thereafter cultures were kept at 37°C in a roller wheel turning at ¼ rpm. Eagle's medium with 10% fetal calf serum, 50 units of penicillin, 50 μg of streptomycin and 1 μg of amphotericin B (Fungizone) per ml.

Soft agar method. The technique originally described by Macpherson(1) was employed in a slightly modified form. Base layers of 5 ml of medium containing 0.5% Difco purified agar were established in 30 ml disposable screw-capped tissue culture flasks (Falcon plastics). Cells were suspended in medium containing 0.3% agar and superimposed upon the solidified base layer. Two ml of suspension containing 3×10^5 cells were added per flask and incubated at 37°C. Flasks were used in preference to Petri dishes in order to prevent contamination with molds during prolonged incubation periods. Occasionally adjustment of pH of the medium became necessary. This was done by replenishing the gaseous phase with a mixture of 5% CO_2 in air.

Fixation and staining. Cell cultures from tubes were fixed and stained with hematoxylin and eosin by employing the collodion embedding technique.(3)

Immunofluorescence staining technique. The indirect test for detection of T ("tumor") antigen was employed(4). Sera from tumor-bearing hamsters were prepared in this laboratory by Dr. Diamandopoulos as previously described(5). A fluorescein labeled anti-hamster globulin rabbit serum was obtained commercially.

Results. The results of 4 analogous experiments will be described in which cells of various species and tissue origin were used. Two experiments were performed with hamster embryo cells, one with newborn hamster lung cells and one with human kidney cells.

Hamster embryo cells. Soft agar. One week after exposure to virus a difference in growth was apparent. Although the initial number of cells in all flasks was the same, more surviving

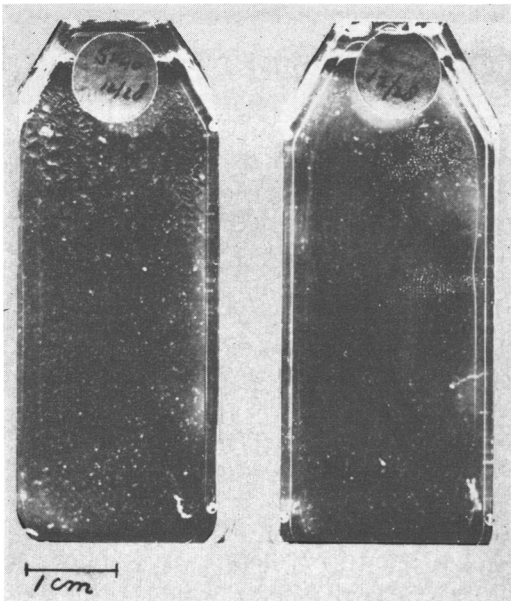


FIG. 1. Growth in soft agar of freshly trypsinized hamster embryo cells. After 3 weeks. Left: SV_{40}-exposed cells; Right: control cells.

and growing cells were present in the flasks containing SV_{40}-exposed cells than in flasks containing control cells. After a total period of 3 weeks these differences became very pronounced as shown in Fig. 1. Many more small colonies were present in the flasks containing SV_{40}-exposed cells. Averages of the number of colonies per field, calculated by counting the colonies in 10 fields from each set of cultures, under a magnification of 100 ×, were: SV_{40}-exposed 9, and control cells 0.4. In a second experiment there was again a difference in the number of colonies observed, although this difference was smaller than in the first. SV_{40}-exposed cells exhibited approximately 7 colonies per field on the average and control cells 2 colonies per field. As stated previously in this experiment smaller embryos were employed. Otherwise the procedure was the same. It is possible that the use of less differentiated cells from younger embryos with greater intrinsic growth potential may be responsible for the quantitative difference in the results.

Morphologically two types of colonies were easily distinguishable both in the flasks with normal and in those with SV_{40}-exposed cells. One was sharp-edged, more translucent and uniform than the other, which was more irregular in outline, more refractile and showed a tendency to break up into smaller aggregates.

Monolayer cultures. Cultures were fixed and stained with hematoxylin and eosin 7, 14, 21 and 30 days following exposure to virus. After 7 days an occasional multinucleated giant cell was observed among the SV_{40}-exposed cells. In these cultures an increased growth activity was apparent as indicated by a higher cell population and more mitotic figures. This early enhancement of growth was strikingly apparent when cultures were initiated with a comparatively small number of cells (± 50,000 cells per tube). Even after 3 days many more cells were present in the cultures of SV_{40}-exposed cells as illustrated in Fig. 2.

During the second week of incubation the number of multinucleate giant cells increased slightly and after 20-30 days of incubation extensive foci of multinucleate giant cells appeared suddenly in the SV_{40}-exposed cell cultures (Fig. 3). Only a few of these foci (ranging from 1 to 5) were seen in each culture. At the time these foci developed, a small percentage (about 1-5%) of the cells were found to be producing T antigen.

Newborn hamster lung cells. Soft agar. During the first 2 weeks no differences in appearance and growth of control and SV_{40}-exposed cells were noted. Approximately the same numbers of virus-exposed and control cells survived in each system. However, during the next 2 weeks most of the control cells failed to multiply and eventually disintegrated, while moderate numbers of SV_{40}-exposed cells continued to survive and divide. At this time pH of the medium in the flasks with SV_{40}-exposed cells was lower. After a total incubation period of 4 weeks approximately 30 colonies such as those shown in Fig. 4 were seen in each flask containing SV_{40}-exposed cells. In addition to these colonies many single surviving cells were noted. In the flasks with control cells no colonies developed although an occasional cluster of single surviving cells was present.

Monolayer cultures. Cultures of lung cells have now been observed throughout 2 months.

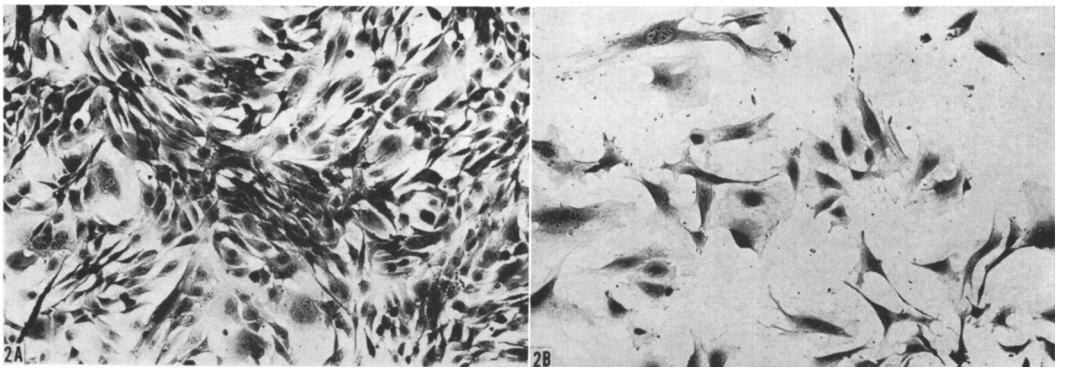
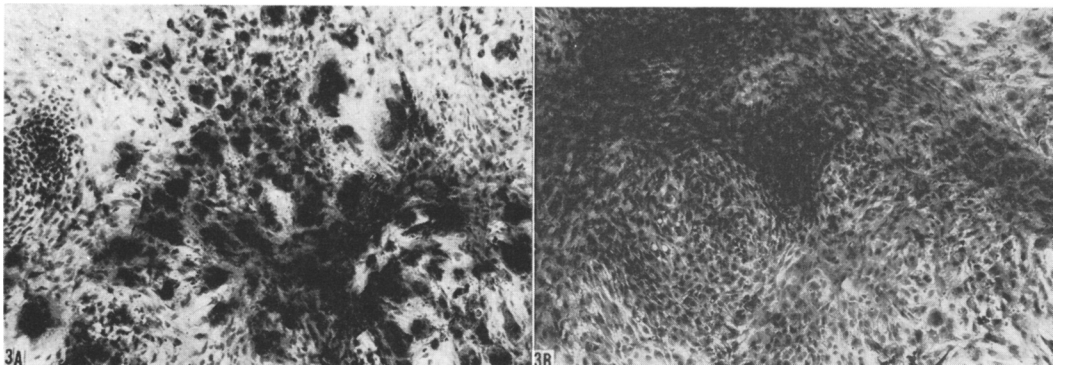


FIG. 2. Cultures of SV_{40}-exposed and control primary hamster embryo cells both initiated with 50,000 cells per tube. After 3 days. A) SV_{40}-exposed; B) control. Magn. × 430.



3. Cultures of SV_{40}-exposed and control primary hamster embryo cells. After 3 weeks. A) SV_{40}-exposed; B) control. Magn. × 430.

Representative cultures were fixed and stained at 3, 5 and 8 weeks. No clearly apparent stimulation of growth was seen in virus-exposed cells. No foci of multinucleated giant cells like those developing in hamster embryo cultures were observed. No T antigen was detected in cells of SV_{40}-exposed cultures in examinations made 3, 5 and 8 weeks after exposure.

Human kidney cells. Soft agar. No growth was observed in flasks with either SV_{40}-exposed or control cells which after persisting for a time slowly disintegrated.

Monolayer cultures. No difference in growth rate between SV_{40}-exposed and control cells was noted during the first 6-9 weeks. Thereafter an increase in number of mitotic figures and a disordered multilayering of fibroblastoid cells appeared in the SV_{40}-exposed cultures as previously described in this cell system(6), and more nuclei with abnormal distribution of chromatin were found. At 8 weeks in a small proportion (*ca.* 5%?) of the cells T antigen was detected.

Discussion. Study of the early responses of cells exposed to SV_{40} have so far been largely neglected. Only in the case of the renal cells of the monkey which are usually destroyed by the virus and do not undergo subsequent transformation and in the case of established cell lines (*e.g.,* BHK21 line of Stoker) have such investigations been reported. So far as we are aware, no attempts have been made to determine *in vitro* the response of cell populations obtained directly from tissues of the animal in which transformation may later occur and which are more nearly representative of the range of cell types and of their biologic status as they exist within the living animal.

The experiments described here were undertaken to provide a technical approach by

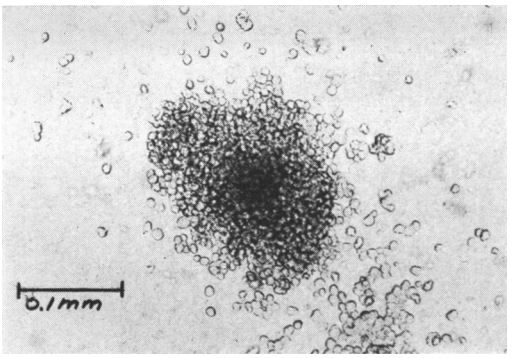


FIG. 4. Colony of SV_{40}-exposed newborn hamster lung cells in soft agar. After 4 weeks.

which such information might be obtained. Although limited numerically and obviously of a preliminary nature, the results are sufficient to indicate that mixed populations of cells derived directly from normal hamster and human tissues react in different ways after exposure to SV_{40}. In soft agar cultures growth of whole hamster embryo cells is rapidly stimulated, that of newborn hamster lung cells is increased after an early lag period, while multiplication of human kidney cells is not affected during the same period of observation. Under these conditions the stimulating effect of the virus in the two hamster cell systems is most strikingly shown by increased capacity to form colonies of virus-exposed cells as compared with controls. With the hamster embryo cells the difference was one of degree; with the hamster lung cells it was absolute. Additional impressive divergencies exhibited by virus-exposed whole hamster embryo and lung cells consisted in the failure of lung cells to produce T antigen and to form multinucleated giant cells in monolayer cultures. The early behavior of exposed lung cells is consistent with published observations(5) on the enhanced survival potential but prolonged delay in morphological transformation, acquisition of oncogenic potential and absence of T antigen in such lung cells treated with SV_{40}.

Our findings raise a number of interesting questions that the procedures here described may possibly help to answer. Some of these are as follows. Do the cells which develop colonies in soft agar represent a small proportion of the total population that is potentially capable of transformation by the virus? Is penetration of the virus into the cell necessary for stimulation of cell division or will mere contact at the cell's surface suffice? What is the nature of the biochemical systems that are affected by the virus which are responsible for increased cell division in certain cells and not in others? In attempts to answer this last question it obviously would be desirable to determine whether the early enhanced growth rate of SV_{40}-exposed hamster embryo cells is associated with an early induction of cellular DNA synthesis as described by Hatanaka and Dulbecco(7) in African green monkey cells infected with SV_{40} virus.

Our results with the soft agar method are in part consistent with those obtained by Macpherson and his coworkers with polyoma virus(1). These authors noted an enhanced capacity of virus-exposed primary cultures of hamster embryo cells to multiply in soft agar. They did not, however, observe any colony formation in control cell cultures whereas in our experiments a few colonies developed in cultures of control embryo cells. Our use of freshly trypsinized organ cells instead of cells prepared by trypsinization of primary cultures may possibly account for this difference.

Investigations are in progress to elucidate the differences in early response to SV_{40} of various kinds of cells and to define the nature and significance in respect to morphologic and oncogenic transformation of the early growth stimulation of hamster cells. The failure of the virus to stimulate early growth of human kidney cells remains unclear. Possibly their lack of response may be related to their more adult state relative to the hamster cell systems, since they were derived from a subject 3 months of age. Further investigation is clearly necessary to account for this difference.

Summary. Suspensions of cells derived from hamster embryonic and lung tissues soon after exposure to SV_{40} virus exhibited enhanced capacity to multiply in a soft agar medium. Such enhancement was not observed in human kidney cells similarly treated. Production of SV_{40} T antigen was seen in hamster embryo cells after 3-4 weeks and in human kidney

cells after 8 weeks following exposure to the virus but was not observed at 3, 5 and 8 weeks in lung cells maintained under the same conditions.

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Effects of Ouabain on Potassium Contractures in Rabbit Atria.* (31348)

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Niedergerke(1) noted that frog heart ventricles took up extra amounts of calcium (Ca) ions when they were made to contract by treating the tissue with solutions containing either reduced sodium (Na) or increased potassium (K) concentrations. Hodgkin and Horowicz(2) observed that K-contractures in single skeletal muscle fibers were more transient in nature than that of the frog heart. They explained this feature by suggesting that there was only a limited quantity of a precursor, perhaps a Ca compound, which was rapidly depleted during activity of the twich fiber so that early relaxation occurred. Therefore, it would appear that in the contraction of the frog heart Ca is continuously available from transfer through the membrane, whereas in skeletal muscle the activator of contraction or its precursor resides in a cellular site, possibly the sarcoplasmic reticulum.

It has been noted in this laboratory that K-contractures in isolated rabbit left atria are transient in nature, and can be modified by ouabain. A summary of these studies is presented here.

Methods. Rabbits weighing between 1 and 2 kg were used. Animals were stunned by a blow to the head. Hearts were rapidly removed and left atria dissected free. The preparations were attached to platinum-iridium electrodes and suspended in 30 ml of a Ringer solution of the following composition (mM): NaCl, 154; KCl, 4.0; $CaCl_2$, 2.3; $NaHCO_3$, 2.4; and glucose 5.6. The solutions were vigorously aerated with O_2 and all experiments were carried out at 30°C. At this temperature contractile tension remains stable for long periods of time (up to 8 hours). Atria were then attached to Statham force displacement transducers and 2 g resting tension applied. Changes in isometric tension were recorded using a Grass polygraph. Atria were stimulated at a rate of 60 cycles/min with 20 volt square wave pulses of 1 msec duration only during the period they were in normal Ringer solution.

Preparations were first equilibrated for 1 hour in Ringer solution and then treated with K_2SO_4-Ringer solution of the following composition (mM) for 30 minutes: K_2SO_4, 116; $KHCO_3$, 2.4; $CaCl_2$, 2.4; and glucose 5.6. In the ouabain studies, atria were incubated an additional 15 minutes in Ringer solution containing the drug at a final concentration of 5×10^{-7} M before placing them in high-K solutions. At this time contractile tension had increased approximately 20%. This concentration of ouabain is in the therapeutic range for rabbit atria.

Some observations on Ca^{45} uptake and tissue Ca content were made during the first 6 minutes of the K-contracture and compared

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