it would appear that the lactate is being channeled to glucose formation rather than to oxidation.

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## Transformation of Hamster Embryo Cells *in vitro* by Murine Sarcoma Virus (Harvey).* (32326)

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Harvey(1) reported the induction of tumors in a variety of rodents by an agent which was isolated from a rat inoculated with Moloney leukemia virus. Chesterman *et al*(2) described the pathology of tumors and other lesions induced in mice, rats and hamsters by the same virus.

Moloney(3) later described the isolation of a virus which induced rhabdomyosarcomas in mice. Huebner *et al*(4) have shown that the Moloney isolate is capable of inducing fibrosarcomas in hamsters which unlike the mouse tumors, do not contain infectious virus. Evidence of the presence of a defective viral genome in the hamster tumor cells was presented(4). The *in vitro* transformation of mouse embryo cells(5) and rat embryo cells (6) by the Moloney sarcoma virus and of mouse embryo cells by the Harvey virus(7) has been described.

For convenience the agents have been termed "Murine Sarcoma Viruses" (MSV) and designated MSV (Harvey) and MSV (Moloney), respectively.

This communication describes the *in vitro* transformation of hamster embryo cells by MSV (Harvey).

*Materials and methods.* Virus. The source of the MSV and the initial transformation of mouse embryo cells has been described(7).

*Cell culture techniques.* Hamster embryo cell cultures were prepared and maintained by the same methods as previously described for mouse embryo cells(7). MSV preparations were assayed by determining the number of foci of transformed cells produced in cultures of Swiss T-O mouse embryo cells. Five hundred thousand mouse embryo cells per

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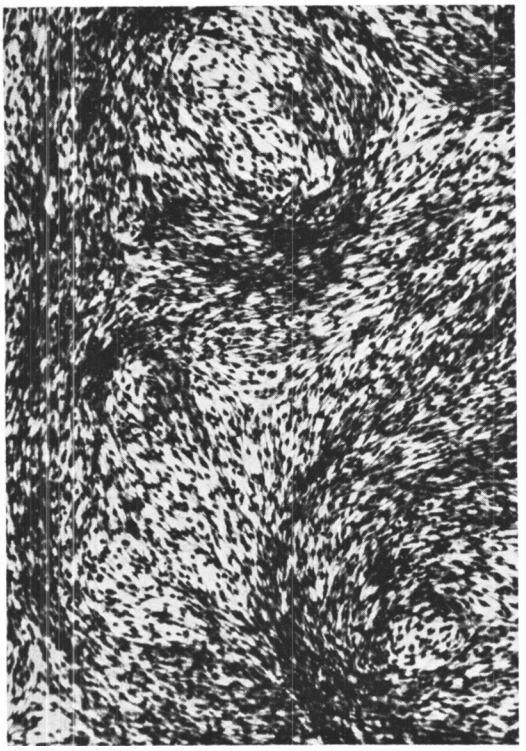

FIG. 1. Mouse embryo cells transformed by MSV and maintained in culture. May Grünwald-Giemsa stain (47×).

60 mm Falcon plastic petri dish were infected in suspension with 0.1 ml of virus dilution. The medium was changed after 2 days incubation, and the dishes were stained with May Grünwald-Giemsa on the fifth day. The numbers of foci were counted using a low power dissecting microscope. This is essentially the same technique as that described by Hartley and Rowe(5).

A line of C57 Black mouse embryo cells transformed by MSV (Harvey) 3 months previously was maintained in this laboratory. These cells grew very vigorously and formed thick multi-layered whorls (Fig. 1). MSV was continuously released into the culture media; which were used as the source of virus for these experiments. To increase the virus titer, tissue culture fluids were centrifuged at 25,000 r.p.m. for 60 minutes in the size-30 head of an M.S.E. high-speed centrifuge and the pellets resuspended in a small volume of culture medium. By this means the virus was concentrated to a titer of $7.5 \times 10^5$ focus forming units (ffu) per ml.

To ensure that infective culture fluids were cell-free, they were filtered through HA (0.45 $\mu$) Millipore® filters. For transformation studies of hamster embryo cells, $10^6$ cells in 4 ml growth medium were infected in suspension with 0.2 ml of virus dilution, and grown in 60 mm plastic petri dishes. The medium on the cultures was changed at 2-3 day intervals. When areas of transformed cells were noted, 1 culture from each group, including a non-infected control, was trypsinized and the cells divided between 3 new dishes. Cultures were then passaged as necessary, usually at 5-7 day intervals.

*Tumor extracts.* Portions of tumor were frozen immediately upon collection, triturated with sand in a chilled mortar and made up to a 10% suspension in cold culture medium. After centrifugation at 2,000 r.p.m. for 10 minutes, the supernatant was collected and either inoculated immediately onto mouse embryo cells or stored at —70°. To test for infectious virus 0.1 ml of tumor extract was added to each of 2 cultures containing $5 \times 10^5$ mouse embryo cells. After 5 days incubation the cells from 1 dish were split. The incubation of a duplicate dish was continued for a further 5 days, when samples of the culture fluid were inoculated onto fresh mouse embryo cells. If none of these cultures showed transformation after 10 days incubation, the tumor was considered to be free of MSV infectious for mouse embryo cells.

*Animals.* Syrian golden or albino hamsters were used from a closed, but not deliberately inbred, colony at the Imperial Cancer Research Fund Laboratories at Mill Hill.

Random bred Swiss T-O or inbred C57 Black mice were used as a source of mouse embryo cells.

*Results. Absence of MSV in hamster tumors induced with mouse-grown MSV.* Repeated attempts to detect infectious MSV in tumors induced by inoculation of new-born hamsters with mouse tumor extracts or culture fluids from MSV-infected mouse embryo cells have been unsuccessful. In another study by one of the authors (J.J.H.), extracts of primary MSV-induced hamster tumors as well as culture fluids from hamster tumor cell cultures were consistently negative for

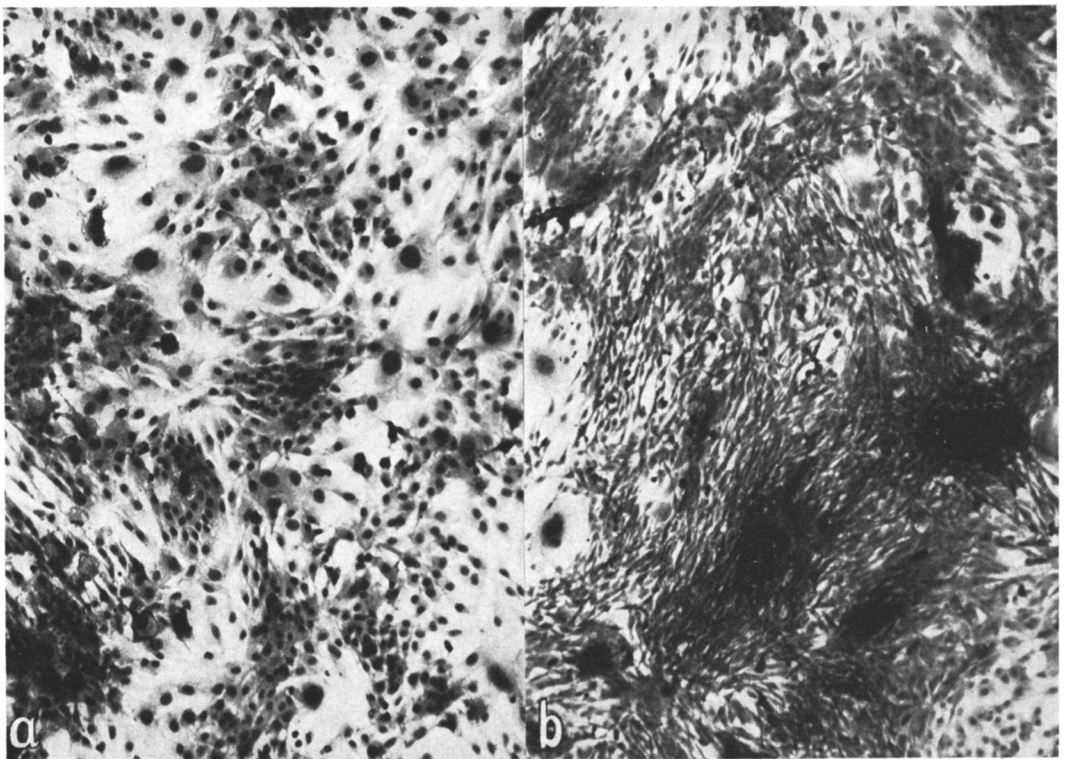


FIG. 2. (a) Control hamster embryo cells. May Grünwald-Giemsa stain (67×); (b) Focus of MSV-transformed hamster embryo cells. May Grünwald-Giemsa stain (60×).

tumor formation in mice.

*Transformation of hamster embryo cells.* In cultures of hamster embryo cells infected with concentrated MSV, foci of densely staining spindle-shaped cells were first noted 6 days after infection (Fig. 2b). There were several areas of these cells, and after a further 2 days incubation the entire sheet had been converted to spindle-shaped cells. On subculture the spindle cells grew vigorously, while uninfected hamster embryo cells retained their normal morphology after 4 passages and 18 days in culture (Fig. 2a). Filtered culture fluids from both transformed and control cultures were inoculated onto C57 Black mouse embryo cells. The mouse embryo cells inoculated with the fluid from the transformed cultures developed small foci of transformed cells after 3 days incubation, and the changes spread rapidly throughout the cultures. Cultures inoculated with control fluids were unchanged after 6 days incubation.

In another experiment hamster embryo cells were infected with serial 10-fold dilutions of concentrated MSV. Cultures infected with undiluted virus contained foci of transformed cells after 6 days, and the changes rapidly spread throughout the cultures on continued incubation. One culture out of three infected with $10^{-1}$ dilution showed a single focus after 6 days incubation, and a number of foci were present in all dishes of this group after a further 2 days. These changes rapidly spread throughout the cultures. Hamster embryo cells infected with higher dilutions of virus ($10^{-2}$ to $10^{-5}$) retained their normal morphology through 7 transfers and 35 days in culture. Aliquots of culture fluids were taken at intervals, filtered and inoculated onto mouse embryo cells. Infectious virus was detected only in fluids taken from cultures showing transformation. Fluids taken from hamster embryo cultures treated with concentrated virus did not contain infectious virus when tested 4 days after infection, but were positive when checked 6 days after infection, at which time foci of transformed cells were present. Control cultures or cultures treated

with dilutions of virus that did not cause transformation did not release detectable virus into the medium.

The results of assays of infectious virus obtained from transformed hamster embryo cells were difficult to interpret, because focus counts did not correspond to virus dilutions. However, dilutions of virus that gave a count of 12-20 foci per plate indicated a titer of at least $5 \times 10^2$ ffu per ml.

Transformed hamster embryo cells grew vigorously and were easily maintained, apparently releasing infectious virus into the medium continuously.

Filtration of the original MSV inoculum through a 0.45 $\mu$ membrane filter should have precluded contamination of these infected hamster cell cultures with transformed mouse cells. Examination of chromosome preparations of colchicine inhibited transformed cells confirmed their hamster origin.

*Animal inoculation with 'hamster-grown' virus.* Filtered fluid from transformed hamster embryo cells was inoculated into new-born C57 Black mice and new-born hamsters. The mice developed multiple tumors very quickly (17-21 days), and postmortem examination showed fleshy spleens enlarged 3-4 times. Virus infectious for mouse embryo cells was easily detected in these tumors.

Hamsters inoculated as new-born animals developed tumors after a latent period of 38-56 days. Surprisingly, virus infectious for mouse embryo cells was easily detected in extracts of these hamster tumors. Fragments from one of the tumors were trypsinized, and a line of hamster tumor cells was established. These cells continuously released virus into the culture medium.

One of these hamster tumors was transplanted into young adult hamsters and MSV was detected in the two tumors tested of this first transplant generation.

*Discussion.* The failure to detect infectious MSV (Harvey) in hamster tumors induced with virus prepared from mouse tumors or mouse tissue cultures confirms previous findings(2). Since MSV (Harvey) readily induces tumors in hamsters, it is not surprising that hamster embryo cells are transformed *in vitro* by the virus. The foci consist of several layers of densely staining, elongated, spindle cells with a few round, pycnotic cells scattered throughout the focus. This is the same appearance as that described for transformed mouse embryo cells(7). However, the hamster embryo cells are much less susceptible to transformation than are mouse embryo cells. At a multiplicity of infection of 0.1, in terms of ffu for mouse cells, transformation was not seen in the hamster cell cultures until 6 days after infection. At the same multiplicity of infection mouse embryo cell cultures showed marked transformation within 48 hours of infection. At a multiplicity of infection of 0.001 ($10^3$ ffu of virus per dish), hamster embryo cells retained their normal morphology until they were discarded 35 days after infection. Thus the transforming efficiency of the virus is reduced at least a thousand-fold in hamster cells as compared to mouse cells. Although the first appearance of transformed cells was delayed, once they appeared in the hamster cell cultures the entire sheet of cells was rapidly involved. These changes seemed too rapid to be the result of an overgrowth of normal cells by transformed cells and were suggestive of the spread of MSV through the cultures. It was confirmed that MSV infectious for mouse embryo cells was present in fluids of hamster cultures showing foci of transformation. Virus was not detectable in the culture fluids until transformed cells appeared. Virus was always detectable in the fluids from cultures of transformed hamster embryo cells maintained by transfer over a period of several months, indicating that the virus was continuously released. When 'hamster grown' virus was assayed on mouse embryo cells, the number of foci did not correspond to virus dilution, being less than that expected at higher dilutions. This same phenomenon was described for MSV(Moloney) by Hartley and Rowe (5). 'Hamster grown' virus was capable of inducing tumors in hamsters, but unlike tumors induced with 'mouse grown' virus, infectious MSV was readily isolated from the tumor cells.

Hartley and Rowe(5) have shown that MSV (Moloney) requires the presence of a mouse leukemia virus to transform mouse

embryo cells *in vitro*. Moreover, the cells of tumors induced in hamsters by MSV (Moloney) carry a defective viral genome that can be 'rescued' by co-cultivation of the hamster tumor cells with mouse embryo cells in the presence of a murine leukemia virus (4). The antigenicity of the "rescued" sarcoma virus is determined by the particular leukemia virus used as a "helper" virus in the system.

Our results suggest that infection of hamster cells with high concentrations of MSV may result in the selection of a non-defective sarcoma virus capable of replicating in hamster cells. However, the complex dose-response curve of hamster grown MSV in mouse embryo cells would not support this hypothesis. Alternatively, a "helper-virus" capable of replicating in hamster embryo cells may have been selected. These preliminary results indicate the need for a detailed comparison of mouse and hamster grown MSV.

*Summary.* Preliminary studies on the transformation of hamster embryo cells by a murine sarcoma virus are reported. Although hamster embryo cells are readily transformed by high concentrations of this virus, they are much less sensitive than are mouse embryo cells. The transformed hamster cells are spindle-shaped and stain very densely with May Grünwald-Giemsa. They grow vigorously and are easily maintained *in vitro* with continuous release of virus into the culture medium. The hamster grown virus shows a complex dose-response curve when assayed on mouse embryo cells. Virus-producing tumors are induced by inoculation of the hamster grown virus into newborn hamsters.

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## Production of Free Light Chains of Immunoglobulin by a Hematopoietic Cell Line Derived from a Patient with Multiple Myeloma.* (32327)

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A number of hematopoietic cell lines have been derived from biopsy tissue of patients with Burkitt's lymphoma(1,2,3,4) and from the buffy coat of patients with various leukemias and lymphomas(5,6,7,8), other malignancies(9), and of normal individuals(10). Many of these cell lines produce various classes of immunoglobulins or their component polypeptide chains(11,12,13). Cultured cells and culture media generally showed reactions with antibodies specific to both heavy chains and light chains of human immunoglobulin, indicating that whole globulin molecules are synthesized in these cultured cells. However, some indication that the cells do not always synthesize equivalent amounts of heavy and light chains was obtained from the results of quantitative radiolabeled antibody technique(12). Indeed, light chains in free form were observed together with whole globulins in products of some of the cell lines(13, and unpublished results).

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