Replication of Murine Sarcoma Virus-HARVEY (MSV-H) in Tissue Cultures of Virus-induced Sarcomas

By JENNIFER J. HARVEY*

Cancer Research Department, The London Hospital Medical College, Whitechapel, E. 1, England

(Accepted 1 May 1968)

SUMMARY

Sarcomas induced in 13 BALB/c mice, one Sprague-Dawley rat and five Syrian (golden) hamsters by the murine sarcoma virus-HARVEY (MSV-H) were grown in cell culture and the cell lines maintained for a maximum of 194 days. The majority of the tumour lines formed three-dimensional colonies of densely packed cells which eventually broke free from the glass and floated in the medium. Infective MSV-H was obtained from the mouse and rat tumour cultures. Filtrates of the culture fluids induced typical MSV-H lesions when injected into newborn mice, and those from cultures maintained for 4 to 6 months were as effective as those from cultures maintained for shorter periods. The newborn mice receiving undiluted culture fluids rapidly developed sarcomas and erythroblastic splenomegaly, while recipients of limiting dilutions developed lymphocytic leukaemia which never appeared earlier than 2 months after injection. Thus, the MSV-H derived from cell cultures behaved in the same way as that derived from infected animals.

However, hamster tumour-cell culture filtrates did not contain active MSV-H when tested in mice, but one such filtrate induced typical sarcomas and other characteristic MSV-H lesions when injected into newborn hamsters.

INTRODUCTION

The isolation, physical properties and preliminary accounts of the pathological effects of the murine sarcoma virus-HARVEY (MSV-H), have been described (Harvey, 1964; Mahy, Harvey & Rowson, 1966; Chesterman et al. 1966). Moloney and Kirsten have also isolated sarcoma-inducing viruses (Moloney, 1966a; Kirsten & Mayer, 1967), but the exact relationship of these three virus isolates to each other has yet to be established.

MSV-H was originally isolated from the cell-free plasma of a rat inoculated at birth with Moloney (1960) leukaemia virus (MLV). When injected into newborn mice, rats and hamsters MSV-H induces the rapid formation of sarcomas at sites dependent on the route of injection, haemorrhagic lesions and cysts, pleural effusions and, in mice and rats, massive enlargement of the spleen (due to proliferation of erythroblasts) which is usually accompanied by a severe anaemia. However, some mice and rats receiving small doses of MSV-H do not show these early effects (i.e. tumours and erythroblastic splenomegaly) but develop lymphocytic leukaemia after periods of 2 months or more (Harvey, 1964). Such recipients show only a large thymic

lymphoma or gross enlargement of the thymus, spleen and all the lymph nodes. The late splenomegaly after small doses of MSV-H is due mainly to proliferation of lymphoblasts and should not be confused with the early erythroblastic splenomegaly. Anaemia also occurs in the leukaemic animals but is not as severe as that which accompanies the early MSV effects. Cell-free preparations of the tissues of such leukaemic mice or rats cause only lymphocytic leukaemia when injected into newborn recipients; there is no recurrence of the early tumours and erythroblastic splenomegaly.

The experiments in cell cultures of Hartley & Rowe (1966) and Huebner et al. (1966) indicated clearly that the Moloney isolate of MSV (MSV-M) is a complex of two viruses, one of which is probably Moloney leukaemia virus which can act as helper in the rescue of the murine sarcoma virus genome present in apparently non-producing hamster tumour cells induced by MSV-M. However, evidence for the presence of MLV in mice infected with MSV-M (as demonstrated by the development of lymphocytic leukaemia) is conflicting (Moloney, 1966b; Lasneret, 1967). Bassin et al. (1968) reported similar rescue of MSV-M from hamster tumour cells by MLV. MSV-M will transform mouse or rat embryo fibroblasts in culture (Hartley & Rowe, 1966; Ting, 1966; Bernard, Boiron & Lasneret, 1967), and MSV-H has also been shown to transform mouse or hamster embryo fibroblasts with the continual release of virus (Simons et al. 1967; Simons, Bassin & Harvey, 1967).

In the experiments to be described long-term cultures of sarcomas induced by MSV-H in mice, rats and hamsters were used. It was hoped to obtain further evidence of the composition of MSV-H, which could be either a single entity or a complex of two viruses, one causing the early, the other the late pathological effects described. The presence of a third erythroblastosis virus such as Friend leukaemia virus (FLV) or Rauscher leukaemia virus (RLV) present as a contaminant was also considered. It was thought that a long-term tissue culture system might preferentially support the replication of one component and hence lead to a separation of viruses, particularly since FLV and RLV are difficult to maintain in cell cultures.

**METHODS**

**Animals**

BALB/c mice and Sprague-Dawley rats were obtained from the Texas Inbred Mouse Co. (TIMCO), Houston, Texas, and from colonies maintained at the Cancer Research Department of the London Hospital Medical College. Syrian hamsters (*Mesocricetus auratus*) from TIMCO, and from the Imperial Cancer Research Fund Laboratories, Mill Hill, London, were also used.

**Virus**

Murine sarcoma virus *harvey* (MSV-H), isolated as previously described (Harvey, 1964), has been maintained in newborn mice or rats by passage of filtered plasma or saline extracts of tumour or spleen. Whole blood from infected mice or rats was diluted with an equal volume of saline containing 20 i.u. heparin per ml. and centrifuged at 2000 g for 10 min. Tumour or spleen extracts were approximately 10% (w/v) in saline. Virus samples were filtered through disposable HA Millipore filters (0.45 μm) and stored in sealed glass ampoules at −70°.
Replication of MSV-H in sarcoma cultures

Tumour cells

Primary tumours were produced by subcutaneous, intramuscular, or intraperitoneal injection of MSV-H into newborn (1- to 3-day-old) mice, rats and hamsters. Mouse tumours from the diaphragm, thigh muscle, peritoneal wall and subcutaneous tissues were used. The hamster tumours were from the muscles of the back or from subcutaneous tissues, and the rat tumour from the diaphragm.

Tissue culture

Growth medium. This consisted of Eagle’s medium containing additional amounts of vitamins and amino acids, 10% tryptose-phosphate broth, 15% calf serum, neomycin sulphate (5 μg./ml.), and streptomycin (125 μg./ml.). The pH was adjusted to 7.4 with NaHCO₃ using phenol red as the indicator.

Primary cultures. Tumours induced by MSV-H were removed under sterile conditions and coarsely minced with scissors. The tissue mince was washed once with saline, 0.25% trypsin (Difco) in phosphate-buffered saline was added and the mixture, containing approximately 2.5% tissue (w/v), was stirred for 30 to 40 min. at 37°C. An equal volume of growth medium was added to the trypsinized suspension which was then centrifuged at 1000 g for 10 min. Tumour cells were resuspended in 10 ml. of growth medium and transferred to T 30 or T 15 flasks. Good results may also be obtained by suspending the washed tumour mince directly in growth medium without trypsinization.

When some of the tumour cells had become attached to the glass after 24 hr incubation at 37°C, the cell debris was discarded and fresh medium added. Subsequently, the supernatant fluids were replaced when a colour change indicated an acid pH.

Subculture. Tumour cell monolayers were trypsinized for 10 min. at room temperature using 0.1% trypsin in phosphate-buffered saline and sometimes cells remaining attached to the glass were also gently scraped off into the medium. Trypsinized cells were centrifuged at 1000 g for 10 min., resuspended in fresh medium by shaking, and transferred to T 30 flasks (approximately 1 to 2 × 10⁶ cells per flask) or to 8 oz medical flasks (approximately 2 × 10⁶ cells per bottle).

Stained tissue cultures. Dilute cell suspensions in growth medium (1 to 2 × 10⁶ cells per chamber) were grown on coverslips in Sykes-Moore tissue-culture chambers (Sykes & Moore, 1960). The cells were examined daily and photographed either in situ or after the coverslips had been removed, washed in warm saline, dried in warm air, stained with Wright’s stain and mounted.

Preparation of inocula for bioassay

Tumour cell-culture fluids were centrifuged at 2000 g for 10 min. and either filtered through a disposable HA (0.45 μ) Millipore filter, or centrifuged for a further 10 min. at 7000 g and the top two-thirds of the supernatant collected. Newborn mice and hamsters were injected intraperitoneally with 0.1 and 0.2 ml. respectively of these inocula.
Histological preparations

Tissues (e.g. tumours or spleens) from mice, rats and hamsters infected with MSV-\( H \) were routinely fixed in formol-acetic alcohol and embedded in paraffin wax. Sections were cut at 5\( \mu \)m and stained with haematoxylin and eosin. Smears of blood and pleural effusions, and imprints of spleen and liver were stained with May-Grünwald–Giemsa.

Haematological techniques

Heart blood was used for the determination of packed cell volumes by the micro-haematocrit method and haemoglobin values by the cyanmethaemoglobin method.

Control mice

Groups of 20 untreated BALB/c mice were killed at 1, 2, 3, 4, 6 and 8 weeks of age to provide comparative data for the experimental animals. Total body, spleen and thymus weights, packed cell volumes and haemoglobin values were determined. Histological sections of spleen and thymus, smears of blood and imprints of spleen and liver were assessed.

RESULTS

Establishment and maintenance of tumour cultures

One rat, four hamster and thirteen mouse tumour cell lines were established and maintained for 4 to 6 months in culture. Additionally, cell lines from two mouse spleens and one hamster tumour were maintained for 6 to 8 weeks. The tumour cells grew slowly at first but, after trypsinization and subsequent subcultivation, grew much more vigorously, acidifying the medium so quickly that it had to be changed 2 or 3 times a week. Most cell lines were subcultured at weekly intervals, but the rat tumour line grew very rapidly and was subcultured more frequently.

Appearance of cultures

All the tumour cultures, irrespective of species of origin, contained small round cells, spindle-shaped cells, and cells which appeared to be in a transitional stage having features common to both. A few giant cells were also present. These cell types were visible in the fully formed monolayers in which the spindle-shaped cells made whirls on the glass. The refractile rounded cells were densely basophilic, often contained numerous basophilic cytoplasmic granules, usually had eccentric nuclei, and could form structures like pseudopodia. The morphology of the spindle-shaped cells varied; they were sometimes multinucleate, having distinct oval nuclei with several nucleoli and irregular clumps of chromatin. Their cytoplasm often contained basophilic granules and, less frequently, was grossly vacuolated (Pl. 1).

As the number of subcultures of most of the cell lines increased, the rounded cells tended to become more numerous and form aggregates which rapidly increased in size to give distinct three-dimensional colonies that could easily be seen with the naked eye. Eventually, many of these colonies were attached to the glass by a stalk of cells which finally broke releasing the aggregates into the medium.

In order to examine colony formation more closely, dispersed rat tumour cells, sparsely seeded on coverslips in Sykes-Moore tissue-culture chambers, were examined daily and either photographed \textit{in situ}, or after fixation and staining. It could be seen
Two MSV-H-induced hamster sarcoma cell lines grown in Sykes-Moore culture chambers and stained with Wright's stain. (a) Culture containing cells of varying morphology but all with eccentric nuclei and similar chromatin. ×480.
(b) Uni- and binucleate spindle-shaped cells with vacuolated cytoplasm, oval nuclei and distinct nucleoli. ×375.
(c to i) Other transitional forms. ×480. 
(f to i) Detail of uni- and binucleate round cells with eccentric nuclei, distinct nucleolus and pseudopodia-like structures. Note similarity of chromatin pattern in spindle-shaped variants, round cells and ‘stalk’ cell. ×975.

J. J. Harvey

(Facing p. 330)
MSV-n-induced rat sarcoma cell line after 150 days in vitro and 3 days after 27th subculture. Cells grown in a Sykes-Moore culture chamber and stained with Wright's stain.

(a) Early colony formation by adherence of round cells. ×400.

(b) Early colony formation by spindle-shaped cells. ×435.

(c) Linkage of cells by cytoplasmic processes. ×375.

(d) Fully formed colony with core of densely stained round cells. ×375.

(e) Various stages of colony formation. Note cytoplasmic links. ×120

J. J. HARVEY
that round cells which were close together, and often in active mitosis, readily adhered in small clumps either to one another (Pl. 2a), or to adjacent spindle-shaped cells, although groups composed mainly of spindle-shaped cells were also seen (Pl. 2b). Cells which were further apart became linked by long, fine, cytoplasmic processes (Pl. 2c) and appeared gradually to coalesce into similar clumps. These small aggregates were apparently the bases of fully formed colonies which consisted mainly of densely stained round cells acting as centres of attraction for surrounding cells (Pl. 2d, e).

Fully formed colonies, which subsequently broke away from the glass and settled in the culture medium, were collected and resuspended. They immediately settled on the glass, acidified the medium within 2-4 hr, and gave rise to sheets of cells which consisted again of round and spindle-shaped cells. These two cell types are probably, therefore, morphological variants of the same cell. The fully formed colonies are probably proliferative; cultures could easily be maintained by successive passage of such colonies without trypsinization.

Table 1. Bioassay in newborn BALB/c mice of filtrates from cultures of mouse and rat sarcomas induced by MSV-N

<table>
<thead>
<tr>
<th>Culture</th>
<th>Subculture no.</th>
<th>Time in culture (days)</th>
<th>No. mice positive*</th>
<th>No. mice injected</th>
<th>Latent period mean and range† (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat tumour (diaphragm)</td>
<td>1-2</td>
<td>36</td>
<td>3/3</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>86</td>
<td>4/4</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>150</td>
<td>4/4</td>
<td>15 (13 to 20)</td>
<td></td>
</tr>
<tr>
<td>Mouse tumour A (peritoneum)</td>
<td>2</td>
<td>59</td>
<td>5/5</td>
<td>44 (31 to 59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>114</td>
<td>6/6</td>
<td>86 (65 to 101)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>194</td>
<td>3/3</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Mouse tumour B (subcutaneous)</td>
<td>1</td>
<td>24</td>
<td>7/7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>126</td>
<td>4/4</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Mouse tumour C (subcutaneous)</td>
<td>5</td>
<td>32</td>
<td>4/4</td>
<td>15 (14 to 20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>124</td>
<td>7/7</td>
<td>19 (17 to 22)</td>
<td></td>
</tr>
<tr>
<td>Mouse tumour D (thorax)</td>
<td>8</td>
<td>97</td>
<td>3/3</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>113</td>
<td>3/3</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>147</td>
<td>12/12</td>
<td>20 (10 to 23)</td>
<td></td>
</tr>
</tbody>
</table>

* All the recipients developed erythroblastic splenomegaly and sarcomas, except the six mice injected with subculture 6 of tumour A, which developed lymphocytic leukaemia.
† Range given in parentheses. When all mice died on the same day no range is shown.

Bioassay of culture fluids

Rat and mouse tumours. Cell-free culture fluids from the original and subsequent passages of one rat and four mouse primary tumours rapidly induced typical early MSV-N effects (i.e. multiple tumours, erythroblastic splenomegaly, severely reduced packed cell volumes and low haemoglobin values) in a total of 59 BALB/c mice injected when newborn. The medium from one passage of tumour A, however, induced only lymphocytic leukaemia in the six recipients.

Fluids from tumour lines maintained for as long as 194 days were as effective as those from earlier passages indicating continual replication of MSV-N in the tumour cells (Table 1). Cell-free fluids from another four mouse tumour cultures, and also from two mouse spleen cultures maintained for a shorter time, gave similar results.
Table 2. Dual effect in BALB/c mice of MSV-μ derived from mouse sarcoma cultures

<table>
<thead>
<tr>
<th>Culture</th>
<th>Time in culture (days)</th>
<th>Dilution of culture filtrate</th>
<th>Total no. mice dying</th>
<th>No. mice</th>
<th>Mean latent period and range* (days)</th>
<th>Lymphocytic leukaemia</th>
<th>No. mice</th>
<th>Mean latent period and range (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour E</td>
<td>58</td>
<td>$10^0$</td>
<td>6/6</td>
<td>6</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>90 (84, 97)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^{-1}$</td>
<td>14/14</td>
<td>7</td>
<td>37 (25 to 54)</td>
<td>2</td>
<td>4</td>
<td>94 (83 to 107)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^{-2}$</td>
<td>8/12†</td>
<td>0</td>
<td>—</td>
<td>4</td>
<td>4</td>
<td>111 (98 to 144)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^{-3}$</td>
<td>4/6†</td>
<td>0</td>
<td>—</td>
<td>4</td>
<td>4</td>
<td>93 (88 to 103)</td>
</tr>
<tr>
<td>Tumour F</td>
<td>66</td>
<td>$10^0$</td>
<td>4/4</td>
<td>4</td>
<td>46 (31 to 64)</td>
<td>0</td>
<td>0</td>
<td>93 (88 to 103)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^{-1}$</td>
<td>4/4</td>
<td>0</td>
<td>—</td>
<td>4</td>
<td>4</td>
<td>106 (98 to 119)</td>
</tr>
<tr>
<td>Tumour G</td>
<td>96</td>
<td>$10^0$</td>
<td>8/8</td>
<td>8</td>
<td>18 (18 to 22)</td>
<td>0</td>
<td>0</td>
<td>100 (79 to 110)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^{-1}$</td>
<td>11/11</td>
<td>11</td>
<td>31 (17 to 72)</td>
<td>3</td>
<td>3</td>
<td>91 (73 to 103)</td>
</tr>
<tr>
<td>Tumour H</td>
<td>14</td>
<td>$10^0$</td>
<td>5/5</td>
<td>5</td>
<td>30 (25 to 40)</td>
<td>0</td>
<td>0</td>
<td>106 (98 to 119)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^{-2}$</td>
<td>5/5</td>
<td>0</td>
<td>—</td>
<td>4</td>
<td>4</td>
<td>93 (88 to 103)</td>
</tr>
<tr>
<td>Tumour J</td>
<td>46</td>
<td>$10^0$</td>
<td>5/5</td>
<td>0</td>
<td>—</td>
<td>5</td>
<td>5</td>
<td>105 (103 to 119)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^{-2}$</td>
<td>3/4†</td>
<td>0</td>
<td>—</td>
<td>3</td>
<td>3</td>
<td>105 (103 to 119)</td>
</tr>
</tbody>
</table>

* Range given in parentheses. When all mice died on the same day no range is shown.
† Survivors killed healthy at 110 to 144 days.
Replication of MSV-\(H\) in sarcoma cultures

In another series of experiments (Table 2), tumour culture fluids collected at various intervals from an additional five mouse tumour lines were diluted \(10^{-1}\) to \(10^{-8}\) in saline and titrated in a total of 95 newborn BALB/c mice. The most concentrated preparations caused typical early MSV-\(H\) erythroblastic splenomegaly and sarcomas (in 18 to 64 days), while most of the mice which received more dilute preparations developed typical generalized lymphocytic leukaemia (from 73 days after infection). Undiluted culture fluids from one tumour (J) caused only lymphocytic leukaemia in the recipients.

**Hamster tumours.** Filtered tissue-culture fluids taken at various intervals from five hamster tumour lines were injected into a total of 75 newborn BALB/c mice. The recipients were observed for 6 months during which time they developed neither the early nor the late pathological changes typical of MSV-\(H\). However, nine out of 13 newborn hamsters injected with one of these filtrates developed the cysts, pleural effusions and sarcomas characteristic of MSV-\(H\) infection in this species. Mature type-C virus particles typical of the murine leukaemias and similar to those already described in organs of mice infected with MSV-\(H\) (Chesterman et al. 1966) were found by electron-microscopic examination in a sarcoma of one of these hamster recipients. Similar examination of one of the five primary hamster tumours induced by MSV-\(H\) used to initiate the cell lines also revealed type-C virus particles (Dr R. Dourmashkin, personal communication).

**DISCUSSION**

Mouse, rat and hamster tumours induced by MSV-\(H\) were grown in vitro for periods of up to 6 months, when the experiments were terminated. Fully infective MSV-\(H\) was readily recovered from the rat and mouse sarcoma cultures which could thus provide a regular source of virus avoiding the hazards of serial passage in animals or transformation of normal embryo fibroblasts.

A striking feature of the majority of the tumour-cell lines was their tendency to form three-dimensional colonies, apparently as a result of positive attraction between cells rather than by random multilayering due to loss of contact inhibition. However, the exact process of colony formation can only be determined by time-lapse cinematography. There was little doubt that the colonies were proliferative as shown by the subsequent growth of the clumps of cells which became detached into the medium. Presumably this detachment was caused by the increasing number of cells in the colonies and/or their decreased adhesion to the glass. The cultures of tumours induced by MSV-\(H\) appeared morphologically very similar to cultures of tumours induced by Rous sarcoma virus (Šimkovič, Valentová & Thurzo, 1962) and to cells transformed by the same virus in vitro (Svoboda & Chýle, 1963). It is interesting to note that Sanford et al. (1967) also considered that similar morphological effects induced by polyoma virus reflected a proliferative response accompanied by decreased adhesion of cells to glass. Nevertheless, the few MSV-\(H\) sarcoma cultures which did not show these changes, although grown in the same medium, also produced infective MSV-\(H\).

When tested in newborn mice MSV-\(H\) derived in cell cultures from mouse and rat sarcomas behaved exactly like MSV-\(H\) derived from animals causing either erythroblastic splenomegaly with or without tumours, or lymphocytic leukaemia, depending on the concentration used. Preferential growth of a tumour-inducing virus or an
erythroblastosis-inducing virus did not occur. Another observation, namely, that it is always possible to demonstrate tumour-inducing capacity in the tissues of mice which only develop erythroblastic splenomegaly after high doses of MSV-H, supports this finding (unpublished observations). Further, the rescue experiments of Bassin et al. (1968) showed that the MSV-H viral genome present in non-producing hamster tumour cells codes for both sarcomas and erythroblastosis. Such findings negate the possibility that MSV-H is contaminated by an erythroblastosis-inducing virus like Friend leukaemia virus—FLV (Friend, 1957) or Rauscher leukaemia virus—RLV (Rauscher, 1962). In any event, rats infected with these viruses (Mirand & Grace, 1962; Swaen, 1966) do not develop the rapid erythroblastic splenomegaly induced by MSV-H in this species (Chesterman et al. 1966). Thus, all evidence points to the conclusion that MSV-H behaves as a mixture of two viruses, one of which produces tumours and erythroblastosis and a second, always present in higher concentration, which produces lymphocytic leukaemia. Since MSV-H was originally obtained from the plasma of a rat infected with Moloney leukaemia virus it is probable that the virus responsible for the lymphocytic leukaemia is MLV, but definite identification must await more precise immunological tests.

The relationship of MSV-H to the isolates of Moloney (MSV-M) and Kirsten is still unresolved. MSV-M, like MSV-H, originated from MLV-infected donors. It is surprising, therefore, that MSV-M causes only sarcomas and not lymphocytic leukaemia in experimental animals (Moloney, 1966b), although the cell-culture studies of Hartley & Rowe (1966) and the rescue experiments of Huebner et al. (1966) implicate a mixture of two viruses. However, the isolate of Kirsten which produces sarcomas and erythroblastosis arose from a lymphoma-bearing donor not known to be infected with MLV.

The finding that filtered culture fluids from the five primary hamster tumours induced by MSV-M were consistently negative when tested in BALB/c mice requires particular comment. It might logically be concluded that the hamster tumours did not produce virus in culture, particularly as numerous filtrates of plasma or tumour extracts from hamsters infected with MSV-M are inactive when tested in mice (unpublished data). However, the demonstration that newborn hamsters developed characteristic MSV-H lesions when given filtered culture fluids from the one hamster tumour tested showed that the primary hamster tumour cells were not non-producing but, apparently, produced virus which was species specific. This extends the original observation of Bassin et al. (1968), that filtered culture fluids from a line of hamster tumour cells (initially derived by passage in newborn hamsters of tumour cells from a mouse infected with MSV-H) was infective for hamsters but not for mice. Moreover, plasma filtrates from eight hamsters bearing tumour transplants derived in the same way have recently been shown to cause typical tumours in 16/31 hamsters, although 49 BALB/c mice remained healthy during the 6-month observation period (unpublished data). These biological data and the presence of virus particles in the tumour of one of these recipient hamsters and in a primary hamster tumour induced by MSV-H lend support to the original electron-microscopic observation by Dourmashkin (Bassin et al. 1968) that the apparently non-producing cells from a culture of a hamster tumour induced by MSV-H contained type-C virus-like particles similar to those of the murine leukaemias. An analogous situation exists in the Rous sarcoma virus system. Dougherty & di Stefano (1965) showed that some 'non-produc-
Replication of MSV-μ in sarcoma cultures

... Rous sarcoma cells contained virus particles demonstrable by electron microscopy. Further, the species specificity of one strain of Rous sarcoma virus is altered by passage through quail cells either in vivo or in vitro (Rauscher, Reyniers & Sackstede 1964; Vogt, 1967).

This work was supported by a 1-year Rosalie B. Hite Fellowship and by an Institutional Research Grant No. IN-43-G-1, both tenable at the Department of Virology, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, U.S.A., and by a British Empire Cancer Campaign grant to the Cancer Research Department of the London Hospital Medical College, London.

Thanks are due to the administrators of the M. D. Anderson Hospital and to Dr L. Dmochowski (Director) and Dr J. A. Sykes of the Department of Virology.

I also wish to thank Dr M. H. Salaman, Director of the Cancer Research Department, London Hospital Medical College, and Dr June East and Dr R. H. Bassin of the Imperial Cancer Research Fund, London, for helpful advice and discussions. Miss J. Burie and Mrs C. Reinecker gave able technical assistance.

REFERENCES


J. J. HARVEY


(Received 29 February 1968)