The Host Range of bryAN Strain Rous Sarcoma Virus
Synthesized in the Absence of Helper Virus

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SUMMARY

Different L-R 'non-producer' cell lines synthesized one of two types of
RSV(O), distinguishable by host range. RSVα(O) has no known natural
host. RSVβ(O) is infectious for Japanese quails, European pheasants and some
Brown Leghorn, White Leghorn and Reaseheath I × C hybrid chickens. The
host range of RSVβ(O) is distinct from other avian tumour viruses. Host
susceptibility to RSVβ(O) is genetically determined in a complex and as yet
unclear way. It is not correlated with response to the A, B, and C sub-groups
of avian tumour viruses or with presence of the natural antigen which reacts
in the COFAL test: nor is it sex-linked. RSVβ(O) is not markedly oncogenic
in hamsters.

The difference between RSVα(O) and RSVβ(O) did not appear to be a herit-
able property of the RSV particle but depended on the type of host cell in
which the virus was cloned. Synthesis of RSVβ(O) occurred only after passage
through COFAL positive host cells. The possible control of the infectious
properties of RSVβ(O) by a helper virus present in an elusive form in per-
missive hosts is discussed.

A stock of inactivated Sendai virus commonly used for cell fusion was
apparently contaminated with an active avian leukosis virus.

INTRODUCTION

The synthesis of infectious virus by non-producing (NP) cells transformed by the
bryan high titre strain of Rous sarcoma virus (bh-RSV, here called RSV) was demon-
strated by Vogt (1967b) and Weiss (1967). Dougherty & Di Stefano (1965) had pre-
viously observed particles which were morphologically indistinguishable from avian
tumour viruses on the surface of NP cells, and Robinson (1967) showed that these
particles had similar physical and chemical properties to RSV. Since the term NP cells
no longer seemed appropriate Hanafusa & Hanafusa (1968) substituted the term
L-R cells (leukosis virus negative Rous cells).

Before these discoveries the bryan high titre strain of RSV was believed to be
incapable of synthesizing progeny virus in the absence of helper viruses (Hanafusa,
Hanafusa & Rubin, 1963). The transformed cells derived from solitary RSV infection
(i.e. L-R cells) contained the viral genome which could be activated by superinfection
with an avian leukosis helper virus to produce infectious RSV. This RSV possessed
the envelope properties of the helper virus used to activate the L-R cells (Hanafusa,

The interaction between RSV and helper viruses is best interpreted as phenotypic
mixing, which also occurs after mixed infection by two ‘non-defective’ strains of avian tumour viruses (Hanafusa & Hanafusa, 1966; Vogt, 1967a). The helper virus confers a wider host range on RSV and, in addition, the rate of maturation of RSV may be considerably enhanced by the helper virus, in which case the proteins coded by the RSV genome comprise only a small proportion of the phenotypically mixed coat (Vogt, 1967b).

Avian tumour viruses are classified into sub-groups according to their host range, interference and neutralization patterns (Vogt, Ishizaki & Duff, 1967). Host range experiments were made in order to compare RSV produced by L−R cells with other sub-groups and to find susceptible hosts for further research. It will be shown that the virus has a peculiar host range and that there are two kinds of L−R cells, which synthesize infectious or non-infectious RSV respectively. This has also been reported by Hanafusa & Hanafusa (1968) who named non-infectious particles RSVα(O), released by L−Rα cells, and infectious virus RSVβ(O), released by L−Rβ cells. This designation will be adopted here too.

METHODS

Nomenclature. RSV particles which have the same genomes but different coats are referred to as pseudotypes (Rubin, 1965) and the origin of the coat is written in parenthesis, e.g. RSV(RAV 1). RSV produced in the apparent absence of helper viruses is called RSV(O) (Vogt, 1967b).

The phenotype of the host is designated (Vogt & Ishizaki, 1965) by the initial of the host species placed before a bar which is followed by the excluded virus sub-group, a designation borrowed from bacteriophage research. For example, C/A denotes a chicken specifically resistant to sub-group A viruses.

Viruses. BRYAN high titre strain RSV was kindly provided by Dr P. J. Simons in 1965. Rous Associated Virus 1 (RAV 1) was isolated from this stock. RAV 2 and RAV 50 were kindly supplied by Dr P. M. Biggs. Virus stocks were grown in leukosis-free Brown Leghorn cells in vitro. RSV(O) was harvested from L−R line six cells except where otherwise stated. RSV pseudotypes were harvested from L−R line six cells superinfected with the appropriate helper and were thus all derived from the same clone of RSV. Culture media were centrifuged at 3000 rev./min., passed through Millipore AP 25 glass fibre prefilters, freeze-thawed three times, and stored at −70°C.

HVJ strain Sendai virus inactivated with ultraviolet (u.v.) irradiation was kindly provided by Dr J. Svoboda.

Embryos. Chick embryos from the Edinburgh Brown Leghorn strain were supplied by Dr J. G. Carr. White Leghorns were supplied by Appleby Farm Ltd, Ashford, Kent. The highly inbred Reaseheath I, C and W lines and the Sykes line B Rhode Island Reds were kindly provided by Drs P. M. Biggs and L. N. Payne. Japanese quail embryos and adult birds were kindly provided by Mr Lee, Beecham Research Laboratories. Bantams, pheasants and geese were kindly provided by Mr B. Johnson.

Virus assays. In vitro assays. Primary cultures were prepared by trypsinization from 10- or 11-day incubated chicken embryos or an equivalent stage of development for other species, i.e. 12 days for pheasants and geese, 9 days for quails and bantams. Culture and assay methods were the same as described by Levinson & Rubin (1966). All cells were cultivated in medium containing 83 % M 199, 10 % tryptose phosphate broth, 5 % heat inactivated calf serum and 2 % of 2-8 % (w/v) sodium bicarbonate.
RSV(O): Host range

Overlay medium for assay plates contained in addition 0.75% final concentration of Agar. Secondary cultures were seeded with $8 \times 10^6$ fibroblasts in 51 mm internal diameter plastic dishes (Falcon). Six to 18 hr later, the culture medium was removed and the fibroblast monolayer was infected with 0.2 ml. appropriate RSV dilution for 45 min., after which the overlay medium was added. Assays were incubated at 38.5°C in a humidified, 5% CO₂ atmosphere for 6 to 8 days and foci of transformed cells were counted macroscopically (Dougherty & Simons, 1962). Doubtful foci were checked microscopically. Assays in quail cells were usually made in 35 mm. diameter dishes seeded with $3 \times 10^6$ fibroblasts, and were fixed in neutral formol saline and stained with Harris's haematoxylin before counting the foci.

In ovo assays. Virus (0.1 ml.) was inoculated on to the chorioallantoic membrane (CAM) of 11-day embryos through an artificial air space and CAM pocks were counted 8 days later.

In vivo assays. Chickens and quails were inoculated with 0.1 ml. virus in the wing web, which was observed for visible tumours over a six-week period. All tumours appeared within 14 days. Hamsters were inoculated subcutaneously in the intrascapular region with 0.1 ml. virus or L-R cells and observed over a six-month period.

Isolation and maintenance of L-R cell lines. Secondary cultures of $8 \times 10^6$ chick or quail cells were infected with approximately 100 focus forming units of RSV(RAV1), RSV(RAV2) or RSV(O) for 45 min. The cells were then washed and trypsinized, and 1 to $4 \times 10^4$ cells were seeded on to $7.5 \times 10^5$ mouse embryo fibroblasts (secondary cultures from CBA x C57Bl F1 hybrid embryos). Four hr later the medium was replaced with agar overlay medium containing 1% appropriate RAV1, RAV2 or RSV(O) antiserum (Weiss, 1969). After 6 to 7 days L-R cells were isolated from plates containing 5 foci or less by harvesting single foci with trypsin in a narrow Pasteur pipette. Some L-R cell lines derived from Brown Leghorns were passaged on Brown Leghorn fibroblast feeder cultures, others on mouse fibroblast feeders, and L-R cell lines derived from other embryos were passaged on mouse fibroblast feeders. The proportion of L-R cells was kept below 10% total cells for serial passages. To ensure that L-R cells maintained on mouse feeder layers remained avian cells, samples were superinfected with RAV1 or RAV2 and the culture medium was subsequently assayed for the presence of the appropriate RSV pseudotype which was always found.

COFAL (complement fixation avian leukosis) tests for the presence of group specific antigen (Sarma, Turner & Huebner, 1964) were generously done by Mr R. C. Chubb, Houghton Poultry Research Station, by the method of Payne & Chubb (1968).

Treatment of cells with Sendai virus was according to the technique of Enders, Holloway and Grogan (1967), modified for monolayer cultures. Confluent fibroblast monolayers ($2 \times 10^6$ cells/51 mm. diameter dish) were infected with 0.2 ml. undiluted RSV(O) for 20 min. at 38.5°C. When RAV1 was used, about $2 \times 10^6$ infectious units were added for a further 20 min. The medium containing virus was then removed and 400 haemagglutinating units of u.v.-inactivated Sendai virus were added in 0.4 ml. Hanks's saline buffered to pH 7.9. The cultures were placed at 4°C for 20 min. and then at 38.5°C for a further 20 min. after which the Hanks's saline was removed, 5 ml. culture medium was added and the cultures were replaced in the incubator at 38.5°C. After 3 or 6 days the culture medium was harvested for virus assay and the cells were re-plated to estimate the number of transformed cells by focus formation.
RESULTS

Host range in chickens

The sporadic detection of RSV(O) from L-R cells previously reported (Weiss, 1967) was found to be largely due to inconsistent susceptibility of embryos of the Brown Leghorn strain of fowls used for assaying the virus. A study was therefore made of the responses of different fowl strains to RSV(O) in order to find susceptible strains and to compare the host range of RSV(O) with that of known sub-groups of avian tumour virus (Vogt & Ishizaki, 1965; Vogt et al. 1967). In addition to Brown and White Leghorns, the inbred Reaseheath lines I, C and W were chosen as representatives of the C/O, C/A and C/B phenotypes respectively (Payne & Biggs, 1966), and the Sykes line B Rhode Island Reds as C/AB and C/ABC phenotypes (L. N. Payne & P. M. Biggs, personal communication). All embryos used for in vitro host range assays of RSV(O) were also tested for response to RSV(RAV1) and RSV(RAV2) and many to RSV(RAV50), representing the A, B and C sub-groups respectively. (Table I). Only Brown Leghorns and White Leghorns included susceptible embryos. The response to RSV pseudotypes of the A and B sub-groups was consistent with the results of Payne & Biggs (1964, 1966). The host range of RSV(O) was distinct from that of the subgroups of avian tumour viruses hitherto described.

<table>
<thead>
<tr>
<th>Host</th>
<th>Phenotype (to known sub-groups)</th>
<th>Number tested</th>
<th>Number susceptible to RSV(O)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown Leghorn</td>
<td>C/O</td>
<td>89</td>
<td>37</td>
</tr>
<tr>
<td>White Leghorn</td>
<td>C/O and C/B</td>
<td>10</td>
<td>2 (C/O)</td>
</tr>
<tr>
<td>Reaseheath I</td>
<td>C/O</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Reaseheath C</td>
<td>C/A</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Reaseheath W</td>
<td>C/B</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Sykes B, RIR</td>
<td>C/AB and C/ABC</td>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>

* Infected with 0.2 ml. 2 x 10⁻³ dilution of RSV(O).
† Focus count > 5; range 26—too numerous to count.

Table 2. Host range of RSV(O) in fowl other than chickens*

<table>
<thead>
<tr>
<th>Host</th>
<th>Phenotype (to known sub-groups)</th>
<th>Number tested</th>
<th>Number susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bantam</td>
<td>B/O</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Japanese Quail</td>
<td>Q/BC</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>European Pheasant</td>
<td>Ph/B</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Goose</td>
<td>G/AB</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

* Infected with 0.2 ml. 2 x 10⁻¹ dilution of RSV(O).

Host range in other birds

Because none of the domestic fowl strains tested was consistently susceptible to RSV(O), other types of fowl were examined for susceptible strains (Table 2). Japanese quails were examined because Rauscher, Reyniers & Sacksteder (1964) found that passage of Bryan RSV in quails yielded progeny virus infectious for quails but with
decreasing infectivity for chickens, and these authors suggested that the virus might represent non-defective RSV (i.e. RSV(O)). Vogt (1967a) subsequently reported the susceptibility of quails to RSV(O). All quails and pheasants were susceptible to RSV(O) while bantams and geese were resistant (Table 2). The response to the three known subgroups is given as the phenotype.

**Genetic control of host response**

While resistance to RSV(O) is not likely to be caused by an interfering virus because it is not an infectious property (Weiss, 1967), it has been difficult to obtain direct evidence that sensitivity to RSV(O) is genetically controlled. Susceptibility might be due to epigenetic rather than genetic factors. Although the cells used for RSV assays were regarded as fibroblasts, the cultures were, in fact, derived from a variety of tissues and may have included cells which were not fibroblastic in origin. Primary cultures were usually prepared from decapitated embryos whose viscera and skin had been partially removed and susceptibility to RSV(O) may have depended on the presence of a minority cell type which was not always present in the assay plates. Chick embryo cultures tend to become increasingly fibroblastic in composition due both to selection of and to modulation to this cell type. Using Brown Leghorns, a series of experiments was made to investigate whether the sensitivity of embryos to RSV(O) depended on the nature of the assay cells.

1. Four separate embryos were assayed for response to RSV(O) and RSV(RAV1) at successive passages (Table 3). Each embryo was consistently resistant or susceptible to RSV(O), though sensitivity to both RSV(O) and RSV(RAV1) decreased with successive passages.

2. Cells derived from whole embryos, eviscerated embryos, and skeletal muscle tissue alone were assayed after one passage in vitro (Table 4). The characteristic pattern of response to RSV(O) and RSV(RAV1) was found irrespective of the 'purity' of the source tissue, which did not noticeably affect the efficiency of plating either.

Table 3. *Infectivity of RSV on different sub-cultures of cells from the same embryos*

<table>
<thead>
<tr>
<th>Passage</th>
<th>Embryo A</th>
<th>Embryo B</th>
<th>Embryo C</th>
<th>Embryo D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSV(O)</td>
<td>RSV(RAV1)</td>
<td>RSV(O)</td>
<td>RSV(RAV1)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>182</td>
<td>500</td>
<td>197</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>204</td>
<td>450</td>
<td>290</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>166</td>
<td>324</td>
<td>145</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>129</td>
<td>311</td>
<td>136</td>
</tr>
</tbody>
</table>

* f.f.u./0.2 ml. undiluted virus; † f.f.u./0.2 ml. 10⁻⁴ dilution.

3. Cultures were prepared from 28 embryos which were older or younger than the 10- or 11-day-old embryos normally used. The ages ranged from 7 to 17 days incubation. When secondary cultures were challenged with RSV(O) or RSV(RAV1) the pattern of response was characteristic irrespective of embryonic age. All 28 embryos were susceptible to RSV(RAV1) while 12 (43 %) were susceptible to RSV(O). The efficiency of plating of RSV(RAV1) tended to be slightly reduced on cells derived from embryos older than 13 days.
RSV(O) was assayed in Brown Leghorns by three different techniques: focus formation of embryonic fibroblasts in vitro, pock formation on the chorioallantoic membrane (CAM) of fertile eggs, and tumour growth in the wing-web of juvenile birds (Table 5). The proportion of susceptible individuals was similar for all three assay methods.

Thus, the differences of sensitivity of Brown Leghorns to RSV(O) were not caused by variations in the assay conditions. The response to RSV(O) depended on the individual embryo and therefore was presumed to be genetically controlled as for other avian tumour viruses.

Table 4. Infectivity of RSV on cultures of different origin*

<table>
<thead>
<tr>
<th>Number of</th>
<th>Number susceptible to RSV(O)</th>
<th>Number susceptible to RSV(RAV1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>embryos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole, carcass, decapitated</td>
<td>6 4 6</td>
<td>6 6</td>
</tr>
<tr>
<td>Head, viscera and most of skin removed</td>
<td>8 4 8</td>
<td>8 8</td>
</tr>
<tr>
<td>Skeletal muscle only</td>
<td>8 3 8</td>
<td>8 8</td>
</tr>
<tr>
<td>Total</td>
<td>22 9 (41%) 22 (100%)</td>
<td></td>
</tr>
</tbody>
</table>
* Assayed as secondary cultures 4 days after primary trypsinization of 10-day incubated embryos.

Table 5. Response of Brown Leghorn birds, CAMs and fibroblast cultures to RSV(O)

<table>
<thead>
<tr>
<th>Method of assay</th>
<th>Number tested</th>
<th>Number susceptible to RSV(O)</th>
<th>Susceptibility to RSV(O) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>in vitro</em> (fibroblasts)</td>
<td>89</td>
<td>37</td>
<td>41.5</td>
</tr>
<tr>
<td><em>in ovo</em> (CAMs)</td>
<td>41</td>
<td>16</td>
<td>39.0</td>
</tr>
<tr>
<td><em>in vivo</em> (wing-webs)</td>
<td>19</td>
<td>7</td>
<td>36.8</td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>60</td>
<td>40.3</td>
</tr>
</tbody>
</table>

To investigate whether susceptibility to RSV(O) was sex linked, Brown Leghorn embryos were prepared for culture at 11 days incubation by which stage they could be sexed by examining the gross morphology of the gonads. Forty-two embryos were studied, of which 10 out of 23 males and 8 out of 19 females were susceptible to RSV(O). This lack of sex linkage was confirmed by sexing the embryos used for CAM assays.

Although a record was kept of the parents of all Brown Leghorn embryos used for RSV(O) assays, there is little information so far on the segregation of susceptible and resistant individuals. No sires or dams have been identified which produce significant numbers of solely resistant or solely susceptible embryos. Further work using selected parents is in progress.

Quantitative analysis of the response of Brown Leghorns to RSV(O)

Approximately 40% of unselected Brown-Leghorn embryos were susceptible to RSV(O) (Table 1), though all the embryos were susceptible to other RSV pseudotypes. Great variation in the sensitivity to RSV(O) was noticed among those embryos classified as susceptible. Forty-five embryos were infected with the same doses of the same stocks of RSV(O), RSV(RAV1) and RSV(RAV2) (Fig. 1). The response to RSV(O) varied over a tenfold range and had an unusual distribution. There was a clear distinction between susceptible and resistant embryos when counts of less than
5 foci were classified as resistant (Fig. 1a). Five resistant embryos were re-assayed with a more potent preparation of RSV(O) and they still registered as resistant.

The variance of focus counts in assays of the three RSV pseudotypes was analysed. If the focus forming efficiency of each virus were constant for all assay plates and all susceptible embryos, the expected distribution of focus counts would be Poissonian. The observed distributions were therefore compared with Poisson distributions (Table 6a) (Bailey, 1959, p. 74). The distribution of focus counts of multiple assay plates from a single embryo fitted the Poisson distribution in each case. In contrast, the variance when single observations for each embryo were pooled was, for each virus, significantly larger than that expected assuming a Poisson distribution (the data were calculated for the same set of embryos as shown in Fig. 1). The sensitivity of the assays, therefore,

![Fig. 1. Focus forming efficiency of three RSV pseudotypes on forty-five Brown Leghorn embryos.](image)

was a property of the embryo and not of the individual culture plates whose variability could be attributed to sampling errors alone. The titre of RSV(O) assayed on CAMs varied over a similar range to the in vitro assays. The distributions of the embryos in response to RSV(RAV1) and RSV(RAV2) in vitro were similar to those obtained by Dougherty & Simons (1962) for Brown Leghorn CAMs infected with BH-RSV. Since the variance of a Poisson distribution is equal to the mean, the ratio of variance to mean (analogous to a variance-ratio) provides a simple estimate of the ratio of the variance of the observed data to that expected for a Poisson distribution. While the variances of the pooled embryos for all three viruses were significantly greater than expected at the 0.1% level, those for RSV(RAV1) and RSV(RAV2) were only about 4 and 15 times greater respectively, but that for RSV(O) was some 250 times greater (Table 6a).

Bauer & Graf (1969) presented evidence that avian tumour viruses possess two antigenic determinants corresponding to two kinds of cellular receptor sites, and suggested that one of the receptors for sub-group B viruses may be identical to the receptor for RSV(O). One prediction of this hypothesis is that cells which are susceptible to RSV(O) might be more sensitive to RSV(RAV2) than resistant cells. In order to test this prediction the mean focus forming efficiencies of RSV(RAV1) and RSV(RAV2) were calculated separately for embryos susceptible and resistant to RSV(O),
but no significant differences were found (Table 6b). The sensitivity of Brown Leghorns to RSV(RAV1) and RSV(RAV2) was therefore independent of the response to RSV(O). Preliminary experiments using sR-RSV-H, a virus belonging to the newly described sub-group D (Bauer & Graf, 1969), kindly supplied by H. Bauer, suggested that this virus had a higher focus forming efficiency on susceptible cells, which could be eliminated by prior treatment with antiserum to RSV(O) (Weiss, 1969).

Table 6. Response of Brown Leghorns to three RSV pseudotypes

(a) Poisson distribution

<table>
<thead>
<tr>
<th>Virus</th>
<th>Test</th>
<th>Number of assays</th>
<th>Mean focus count</th>
<th>Standard deviation</th>
<th>Variance</th>
<th>mean</th>
<th>$\chi^2 = \text{Sums of square/mean}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV(RAV1)</td>
<td>Pooled embryos</td>
<td>45</td>
<td>189.2 ± 26.8</td>
<td>3.84</td>
<td>1.69</td>
<td>$P &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single embryo</td>
<td>12</td>
<td>192.9 ± 12.9</td>
<td>0.87</td>
<td>9.52</td>
<td>$P &gt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>RSV(RAV2)</td>
<td>Pooled embryos</td>
<td>45</td>
<td>120.5 ± 42.3</td>
<td>1.48</td>
<td>83.0</td>
<td>$P &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single embryo</td>
<td>12</td>
<td>125.3 ± 14.3</td>
<td>1.63</td>
<td>17.86</td>
<td>$P &gt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>RSV(O)</td>
<td>Pooled embryos*</td>
<td>22</td>
<td>475.4 ± 347.1</td>
<td>253.4</td>
<td>5321</td>
<td>$P &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single embryo</td>
<td>20</td>
<td>172.3 ± 13.3</td>
<td>1.03</td>
<td>19.62</td>
<td>$P &gt; 0.05$</td>
<td></td>
</tr>
</tbody>
</table>

(b) Sensitivity to RSV(RAV1) and RSV(RAV2) of embryos which are susceptible or resistant to RSV(O)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Response to RSV(O)</th>
<th>Number of embryos</th>
<th>Mean focus count</th>
<th>Standard deviation</th>
<th>Student's $t$ $\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV(RAV1)</td>
<td>Susceptible</td>
<td>22</td>
<td>197.1 ± 29.1</td>
<td>0.05 &gt; $P &gt; 0.025$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>23</td>
<td>181.7 ± 25.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV(RAV2)</td>
<td>Susceptible</td>
<td>29</td>
<td>123.2 ± 36.0</td>
<td>$t = 0.44$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>28</td>
<td>118.2 ± 48.7</td>
<td>$P &gt; 0.05$</td>
<td></td>
</tr>
</tbody>
</table>

* Susceptible embryos only.
$\dagger$ One-tail test on the assumption that embryos susceptible to RSV(O) may have greater sensitivity to other pseudotypes.

Host range of RSV(O) assayed on chorioallantoic membranes

While fibroblast cultures are most suitable for assaying the response of individual embryos to several virus strains, it is more convenient to screen the response of large numbers of embryos to one virus with the CAM pock assay. The tests reported here were made in collaboration with L. N. Payne, Houghton Poultry Research Station. The results of CAM assays of the pure Reaseheath lines, Brown Leghorns and Rhode Island Reds, were consistent with the host range determined from in vitro assays. However, certain crosses between the Reaseheath I and C lines yielded a proportion of susceptible membranes (Table 7). The segregation of susceptible membranes in the F2 generation and from backcross matings to the C line did not fit any simple genetic segregation patterns. More detailed studies on an individual sire-dam basis of the F2 generation and first and second backcrosses to the C line did not clarify the genetic basis for susceptibility. The proportion of susceptible embryos differed widely, ranging from 8 to 42 % in samples of not less than 24 embryos per mating. No crosses yielded 100 % susceptible offspring and many nominally identical matings gave all resistant embryos. The fact that both parent lines were resistant while many hybrids were susceptible suggested that the response to RSV(O) was controlled by more than one
gene. All embryos were sexed when the membranes were counted and, like the Brown Leghorns, the response of Reaseheath I and C line hybrids was neither sex-linked nor influenced by sex.

Table 7. Host range of RSV(O) in chickens, assayed on the chorioallantoic membrane

<table>
<thead>
<tr>
<th>Type of embryo</th>
<th>Phenotype</th>
<th>No. tested</th>
<th>No. susceptible*</th>
<th>Susceptibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown Leghorn</td>
<td>C/O</td>
<td>41</td>
<td>16</td>
<td>39.0</td>
</tr>
<tr>
<td>I</td>
<td>C/O</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>C/A</td>
<td>145</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td>W</td>
<td>C/B</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sykes line B RIR</td>
<td>C/AB and C/ABC</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I(\delta) x I(\delta)</td>
<td>C/O and C/A</td>
<td>166</td>
<td>30</td>
<td>18.1</td>
</tr>
<tr>
<td>C(\delta) x I(\delta)</td>
<td>C/O and C/A</td>
<td>129</td>
<td>26</td>
<td>20.2</td>
</tr>
<tr>
<td>I(\delta) x I(\delta)</td>
<td>C/O</td>
<td>43</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Pock count > 5 (range: 4-> too numerous to count).

Table 8. Independence of susceptibility to RSV(O) and presence of complement-fixing antigen

<table>
<thead>
<tr>
<th>Type of embryo</th>
<th>Response to RSV(O)</th>
<th>Positive</th>
<th>Negative</th>
<th>Independence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown Leghorn</td>
<td>Susceptible</td>
<td>7</td>
<td>3</td>
<td>P* = 0.99</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>5</td>
<td>2</td>
<td>Not significant</td>
</tr>
<tr>
<td>Reaseheath C x IC</td>
<td>Susceptible</td>
<td>9</td>
<td>2</td>
<td>(\chi^2,^\dagger) = 1.44</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>10</td>
<td>6</td>
<td>Not significant</td>
</tr>
<tr>
<td>Quail</td>
<td>Susceptible</td>
<td>19</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Fisher's exact probability test; † with Yates’s correction.

Relationship between response to RSV(O) and presence of natural complement-fixing antigen

Dougherty & Di Stefano (1966) found that apparently leukosis-free chick embryos contained a complement-fixing antigen for COFAL antisera. The antigen is indistinguishable from the group-specific antigen for avian sarcoma viruses (Dougherty, Di Stefano & Roth, 1967; Payne & Chubb, 1968), which may, however, comprise more than one protein moiety (Duesberg, et al. 1968). Payne & Chubb (1968) showed that the complement-fixing antigen is present in Reaseheath I line embryos but absent from C line embryos, and that its presence is controlled by a single, autosomal, dominant allele. Many Brown Leghorns also synthesize the complement-fixing antigen (R. C. Chubb, personal communication). Both the I line and the C line are resistant to RSV(O), but as some cross-bred embryos are susceptible, the possibility that the presence of complement-fixing antigen might be one of the factors controlling response to RSV(O) was investigated (Table 8). Tissue extracts of 11-day Brown Leghorn embryos and 9-day quail embryos were prepared for COFAL tests at the same time as primary cultures were made; and RSV(O) was assayed in vitro. The Reaseheath embryos were tested at 19 days, following the assay of RSV(O) on CAMs. Although only a few of embryos were tested the response to RSV(O) was clearly independent of the presence or absence of the complement-fixing antigen.
Insusceptibility of hamsters to RSV(O)

The BRYAN strain is not markedly oncogenic for mammals, but tumours may be obtained by inoculation of large doses (Rabotti, Raine & Sellers, 1965; Eidinoff et al. 1965). Its oncogenicity for mammals is strongly influenced by the pseudotype, since Hanafusa & Hanafusa (1966) found that RSV (RAV 50) was oncogenic and RSV (RAV 1) was not. The transformation of mammalian cells following infection with high doses of RSV (RAV 1) may be due to either the large total number of RSV particles or to small quantities of another pseudotype, possibly RSV (O), present in the stock. The response of new born Syrian hamsters to RSV (O) was tested by subcutaneous inoculation with undiluted virus (titre: $10^4$ f.f.u./ml. on quail cells) or with L-R cells. Seventeen hamsters of both sexes which were inoculated with RSV (O) did not develop any tumours during 6 months observation; neither did 6 hamsters inoculated with $10^5$ L-R 6 cells producing RSV (O). Five hamsters were inoculated with $8 \times 10^5$ f.f.u. RSV (RAV 1) and one developed a tumour at 2 months, after which this group was lost. The tumours produced in Syrian hamsters by BRYAN strain RSV were not therefore caused by small quantities of RSV (O).

RSV(O) synthesized by different L-R cell lines

Hanafusa & Hanafusa (1968) found that while some L-R lines produced infectious virus which they called RSV$\beta$(O), others produced particles which were not infectious for quails, called RSV$\alpha$(O). They suggested that BRYAN strain RSV is a mixed population of $\alpha$ and $\beta$ genotypes, both phenotypically masked, usually, by the helper-coded envelope. The experiments described here confirmed the existence of infectious and non-infectious forms of RSV (O), but suggested that these forms were not heritable properties of the RSV (O) particles themselves.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Type of RSV(O) produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-infectious $\alpha$</td>
</tr>
<tr>
<td>BrL</td>
<td>RSV (RAV 1)</td>
</tr>
<tr>
<td>BrL</td>
<td>RSV (RAV 2)</td>
</tr>
<tr>
<td>BrL</td>
<td>RSV (O)</td>
</tr>
<tr>
<td>I-line</td>
<td>RSV (RAV 1)</td>
</tr>
<tr>
<td>C-line</td>
<td>RSV (RAV 2)</td>
</tr>
<tr>
<td>Quail</td>
<td>RSV (O)</td>
</tr>
</tbody>
</table>

* Pseudotype used to obtain L-R line. All pseudotypes derived from same clone of RSV(O).

The RSV (O) described in this report so far was synthesized by one Brown Leghorn L-R line (no. 6). Other L-R lines derived from Brown Leghorn and I line embryos produced infectious virus with the same host range as RSV (O) from L-R 6, and antisera prepared against L-R 6 RSV (O) (Weiss, 1969) neutralized these RSV (O) stocks. A stock of RSV (O) kindly supplied by Dr P. K. Vogt also had the same host range and sensitivity to antiserum. These infectious virus stocks are designated RSV$\beta$(O), according to Hanafusa & Hanafusa (1968). Further L-R lines were derived from
Reaseheath C line cells and quail cells; these lines did not produce infectious virus (Table 9), although the virus particles which initially transformed these L-R lines had been synthesized by the L-R line 6. Non-infectious particles, designated RSVα(O), were synthesized by all the Reaseheath C and quail L-R lines tested. These particles were detectable by electron microscopy of L-R cells; similar numbers of typical virus particles were found for C line L-Rα and Brown Leghorn L-Rβ cell types. A rough estimate of particles in thin sections indicated 1/100 to 1/1000 the number of particles compared to L-R lines superinfected with RAV2.

Non-infectious virus from Reaseheath C line L-R cells was also detectable by treating cells which had been infected with RSVα(O) and RAV1 with Sendai virus inactivated by ultraviolet (u.v.) irradiation (Table 10). This treatment allowed the penetration of particles into resistant cells and resulted in cell transformation and production of helper-coated progeny. Natural resistance to RSVβ(O), RSV(RAV1) and RSV(RAV2) could also be overcome by treatment of C/A and C/AB cells with Sendai virus (unpublished observations).

Table 10. Biological activity of non-infectious RSVα(O) released by C line L-R cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Transformation</th>
<th>Virus progeny at 6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV(O)</td>
<td>-</td>
<td>none</td>
</tr>
<tr>
<td>RSV(O) + RAV1</td>
<td>-</td>
<td>RAV1*</td>
</tr>
<tr>
<td>Inactivated Sendai virus</td>
<td>-</td>
<td>none</td>
</tr>
<tr>
<td>RAV1 + inactivated Sendai virus</td>
<td>-</td>
<td>RAV1*</td>
</tr>
<tr>
<td>RSV(O) + RAV1 + inactivated Sendai virus</td>
<td>+</td>
<td>RSV(RAV1)†</td>
</tr>
</tbody>
</table>

* Assayed by interference of RSV(RAV1).
† Assayed by focus formation.

Table 11. Lack of hosts for RSVα(O)

<table>
<thead>
<tr>
<th>Possible hosts tested</th>
<th>Response to RSV(O)</th>
<th>Response to RSVα(O) produced by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brown Leghorn</td>
<td>C line L-R cells</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>0/8*</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
<td>0/16</td>
</tr>
<tr>
<td></td>
<td>I line</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>26 % Susceptible</td>
<td>0/68</td>
</tr>
<tr>
<td></td>
<td>Sykes B RIR</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>Bantam</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>Quail</td>
<td>1/15</td>
</tr>
<tr>
<td></td>
<td>Pheasant</td>
<td>0/9</td>
</tr>
</tbody>
</table>

* Number susceptible/total number tested.
† Assayed on CAMs. All other hosts assayed in vitro.

No natural host for RSVα(O) was found (Table 11), with the possible exception of one quail embryo whose sensitivity was not repeatable in subsequent assays of frozen preserved cells. The host range of RSV(O) from quail L-R lines is less clear (Table 11); four embryos were susceptible, though the number of foci was small (less than 30) in each case. Two contradictory explanations of this result are possible: (i) that small quantities of RSVβ(O) were synthesized which could be detected only on particularly
sensitive cells; (2) that relatively larger quantities of RSVα(O) were produced which overcame the resistance of occasional assay embryos. Preliminary electron microscopical studies implicated the second explanation.

In addition to the embryos assayed in vitro, three out of six adult quails inoculated with 0.2 ml. undiluted quail RSVα(O) developed tumours within 2 weeks. No tumours developed over 9 weeks observation of five quails inoculated with undiluted RSVα(O) from C line cells, while all seven quails inoculated with 0.1 ml. 10^-3 dilution of RSVβ(O) from Brown Leghorn L-R6 cells developed tumours.

Modification of viral properties by host cell or helper virus?

The infectivity of RSV(O) was correlated with the strain of host cell synthesizing the virus and not with the pseudotype of the original transforming particle (Table 9). The host cell may have determined whether the RSV(O) produced was of the α or β type. If this were so RSV(O) would modulate between α and β characters according to the cell type it is grown in. To test this hypothesis, fibroblast cultures from six separate quail embryos (including two from which L-R clones were derived) were infected with about 1000 f.f.u. RSVβ(O) from L-R6 cells and progeny virus was harvested after 7 and 10 days. RSVβ(O) progeny was always demonstrable when assayed in quail and Brown Leghorn cells. Thus when quail cells were infected with a large dose of RSVβ(O) the progeny bred true for ‘β’ host range, but when the RSVβ(O) was recloned in quail or C line chicken cells it appeared to lose its infectivity and became RSVα(O). These results suggest that ‘β’ may be an infectiously propagated character which, like a helper virus, is not heritably transmitted by single, transforming, RSV(O) particles.

The reverse type of experiment was attempted, using RSVα(O) synthesized by Reaseheath C line L-R8 cells. Since this virus was not infectious it was introduced into Brown Leghorn cells by treatment with u.v.-inactivated Sendai virus. Typical RSVβ(O) was subsequently recovered once, but in two other attempts infectious RSV was obtained which had a wider host range than RSVβ(O), conforming to that of subgroup B avian tumour viruses. This unexpected result was probably due to contamination of the Sendai virus stock by a leukosis virus. The original stock, supplied by Dr. H. G. Pereira, had been grown in eggs which were not guaranteed leukosis-free (J. Svoboda, personal communication). Leukosis viruses are 20 times more resistant to u.v.-inactivation than some myxoviruses (Levinson & Rubin, 1966). These experiments must be repeated using a leukosis-free source of Sendai virus.

Vogt, Sarma & Huebner (1965) demonstrated the presence of group specific antigen in L-R cells and Duesberg et al. (1968) showed that RSV(O) contains the two major proteins of the group-specific antigen found in other RSVs. It is not known whether these cells and virus were β or α in type. COFAL tests were therefore made on Reaseheath L-R α cells and on quail cells infected with RSVβ(O). Both cell types contained group-specific antigen.

DISCUSSION

The host range of RSV(O) produced by leukosis-negative Rous (L-R) cells, formerly known as non-producing cells, was examined. Two types of RSV particle, distinguishable by the host range, were produced by different L-R cell lines. RSVα(O) is not naturally infectious for any known host but was shown to be a competent virus giving rise to cell transformation and virus progeny when the block to infection was by-
RSV(O): Host range

passed. RSVβ(O) was infectious, with a host range distinct from all other avian tumour viruses.

The avian tumour viruses comprise a group of viruses with homogeneous morphological and physical characters and a common group-specific antigen (Vogt, 1965). They have been divided into three major sub-groups, A, B and C, on the basis of host range, antigenic and interfering properties (Vogt et al. 1967). The host range experiments do not reveal any relationship between RSVβ(O) and the A, B or C sub-groups. Vogt (1967b) and Hanafusa & Hanafusa (1968) found that most C/A chickens were susceptible to RSVβ(O) whereas in the host range studies reported here, using different strains of chickens, C/A embryos are resistant to RSVβ(O), but 40% of the C/O embryos are susceptible. No chicken hosts susceptible to RSVβ(O) have been reported which were resistant to sub-group B viruses, but quails and pheasants belong to this category. The Japanese quails used in this study were susceptible to RSVβ(O), resistant to the B and C sub-groups, and variably susceptible to the A sub-group.

Host response to avian tumour viruses of sub-group A (Crittenden, Okazaki & Reamer, 1964; Payne & Biggs, 1964) and sub-group B (Rubin, 1965; Payne & Biggs, 1966) is controlled by single autosomal loci which segregate independently (Crittenden et al. 1967). Susceptibility or resistance is expressed at the cellular level with the allele for susceptibility dominant over the allele for resistance. The response of cells to RSVβ(O) also appears to be genetically determined because it varies with the type of host but not with a variety of different assay conditions. However, the receptors for RSVβ(O) do not appear to be controlled by a single dominant locus; although the inbred I line and C line Reaseheath embryos are resistant to RSVβ(O), some of their hybrids, particularly backcrosses to the C line, are susceptible, indicating the involvement of more than one gene. The wide variation in sensitivity of embryos classified as susceptible may also have a genetic basis.

A fourth class of avian tumour viruses, sub-group D, has recently been identified (Bauer & Graf, 1969; P. H. Vogt, personal communication.) These viruses have a wide host range, infecting C/B as well as C/O and C/A cells, yet are subject to interference by sub-group B and not sub-group C. Sub-group B viruses also interfere with RSVβ(O) (Vogt, 1967b; Weiss, 1969). Sub-group D viruses are oncogenic in mammals but RSVβ(O) is not. The relationship between sub-groups B and D and RSVβ(O) is not clear, and is discussed in the following paper (Weiss, 1969).

Cellular resistance to RSV(O), like resistance to other avian tumour viruses, occurs early in the life cycle of the virus. It may be by-passed by phenotypic mixing with helper viruses to form appropriate RSV pseudotypes or by treating cells with u.v.-inactivated Sendai virus. Piraino (1967) demonstrated that the resistance of C/A cells to RSV (RAV1) is caused by a block to penetration rather than adsorption of the virus. The successful infection of resistant cells by RSV(O) following treatment with inactivated Sendai virus suggests that this virus also can efficiently adsorb to, but not naturally penetrate, the cell surface.

The acquisition of a sub-group B type host range by progeny of RSVα(O) following infection mediated through Sendai virus indicates contamination of the Sendai stock with a leukosis virus—indeed, it is a sensitive assay for such a contaminant. Because most of the commercial sources of eggs used for the growth of Sendai virus are endemically infected with leukosis viruses, live or partially inactivated leukosis viruses have probably been introduced frequently but unintentionally into experiments.
involving Sendai-induced cell fusion. With the increasing use of inactivated Sendai virus for studying changes in cellular and virus metabolism following the formation of hetero-
karyons (Harris & Watkins, 1965; Svoboda, Machala, & Hlozanek, 1967; Watkins &
Dubbeco, 1967) and for infecting unnatural host cells with viruses (Enders et al.
1967), it will be unwise to ignore the probability of contamination with leukemia
viruses. The use of β-propiolactone (Neff & Enders, 1968) may eliminate the differential
inactivation between myxoviruses and avian tumor viruses found with u.v. irradiation
(Levinson & Rubin, 1966) but it will be preferable to obtain clean stocks of Sendai in
the first place.

Bryan strain RSV is clearly not genuinely defective in its capacity to produce
progeny particles, as was formerly thought (Hanafusa et al. 1963). Dougherty &
Di Stefano (1965) showed that L-R cells synthesized morphologically typical particles
whose infectivity was demonstrated by Vogt (1967 b) and Weiss (1967). That infectious
RSV(O) was not recognized sooner (Rauscher et al. 1964) and Siminoff (1964) did
provide suggestive evidence) was probably due to its restricted host range. A further
factor which may have impeded the detection of RSV(O) and which has not yet
received adequate explanation, is the small amount of infectious virus synthesized
by L-R cell lines. It probably corresponds to a low rate of synthesis of physical
particles (Haguenau & Hanafusa, 1968) though Robinson (1967) found that her L-R
cells were not deficient in particle synthesis. It was noted by Hanafusa & Hanafusa
(1968) and in this report that L-β cells and L-Rβ cells produce similar numbers of virus
particles, so the difference between these two types of RSV must be qualitative rather
than quantitative.

The production of two types of RSV(O), α and β, poses more questions than it
answers. RSV β(O) may represent more than one type of virus for it is classified as
present by a negative property, namely, lack of a susceptible host. Five hypotheses may
be proposed to account for the determination of α and β properties of L-R cells.

α and β are heritable characters of RSV(O) and any change is due to mutation or
recombination. Hanafusa & Hanafusa (1968) isolated RSV α(O) and RSV β(O) from
uncloned βt-RSV which they concluded was constituted of a mixture of the two types.
Each variant appeared to be stable in the absence of helper viruses, but when L-Rα cells
were superinfected with RAV 1, about 15 % of the re-isolated RSV clones were β in
type. Those authors suggested that the conversion was due to genetic recombination
between RSV and RAV 1 but if this is so, it is strange that RSV recombinants have
not been isolated which have the full host range and antigenic properties of the helper
virus. In contrast, the experiments reported in this paper were made with cloned
RSV β(O) (from L-R line 6) and the pseudotypes used for re-isolation of L-R lines did
not affect the subsequent character of RSV(O) which was determined by the type of host
cell used for recloning the virus. A preliminary experiment indicated that the reverse
conversion, from RSV α(O) to RSV β(O), also occurred. The difference between my
results and those of Hanafusa & Hanafusa (1968) is difficult to reconcile, although
those authors did not report whether the L-Rα and β lines and sublines were derived
from the same embryos, so that any individuality of the hosts was not taken into
account. Genetic conversion could be tenuously upheld by assuming a high sponta-
naneous mutation rate between α and β, combined with extreme selection of one or the
other type in the host cell. While this seems improbable, mutations should not be
entirely discounted; changes from round cell morphology to mixed round and fusiform
morphologies (Temin, 1960) are commonly observed in L−R lines maintained in this laboratory. Host-range mutants might also occur, but they have not been found even though the passaging of L−R cells on chick fibroblast feeders would inevitably provide a selective system for a suitable mutant.

Host modification of RSV(O). If the production of RSVβ(O) is permitted by certain cell types only, the virus may be dependent on a protein synthesized by that cell type. It is remarkable that the Reaseheath I line and the Brown Leghorn strain, which permit RSVβ(O) production, include COFAL positive embryos. All L−Rβ clones tested are derived from COFAL positive embryos and all L−Rα clones from COFAL negative embryos, but the number of different embryos used so far for L−R isolations is small (8 COFAL positive, 6 COFAL negative) and the association of natural complement-fixing antigen and RSVβ(O) production should not be regarded as conclusive. Since L−Rα cells have become COFAL positive on transformation, RSVα(O) is not defective for the group-specific antigen itself. Modification of avian tumour viruses by the host has been suggested by Hamazaki et al. (1957) and by Shipman & Levine (1966), but these authors did not use cloned virus so that the observed modification may have been due to selection of types or pseudotypes from a mixed stock. The evidence against host modification of RSV(O) is that quail cells, which yield L−Rα cells following clonal transformation by RSVβ(O), are able to synthesize relatively high titres of RSVβ(O) following mass infection.

The permissive cell activates a 'β' gene of the virus. This meets with the same objection as host modification, unless, once activated, the β gene continues to be expressed in both cell types, but there is no explanation of its apparent repression by recloning in non-permissive cells.

A helper virus is present in RSVβ(O) stocks. The apparent influence of the host cell on the type of RSV(O) produced raises the question whether the character is in reality coded by a β type helper virus present in those hosts permitting RSVβ(O) synthesis. This would explain the continued synthesis of RSVβ(O) following multiple infection of quail cells, with conversion to RSVα(O) on cloning. A helper virus in RSVβ(O) stocks was not demonstrable by interference tests (Vogt, 1967b; Weiss, 1969), but this method would only detect a helper present in excess of the transforming virus or one with a fast growth rate. A more sensitive test is to activate RSVβ(O) production in L−Rα cells, which is currently under investigation. An attempt at L−R cell activation by Hanafusa & Hanafusa (1968) probably failed because the L−Rα cells were not susceptible to RSVβ(O) and its putative β helper virus. There is no direct evidence for the presence of a helper virus in Reaseheath I line (Payne & Chubb, 1968) or Brown Leghorn embryos, which continue to permit RSVβ(O) production after recloning, but further investigations, using quail cells as an assay system, are necessary. A congenitally passaged helper virus might be defective itself and undergo mutual complementation with RSVα(O), but complementation between two types of defective RSV genomes, such as that found for host dependent mutants of bacteriophage f2 (Valentine, Engelhardt & Zinder, 1964) or that suggested but not found for defective simian virus 40 lysogens (Dubbs & Kit, 1968), seems improbable because of the failure to isolate L−Rα cells on repeated cloning of RSV in Brown Leghorns.

An integrated helper virus is present in Reaseheath I line and Brown leghorn cells, which complements RSV(O). The possible integration of avian tumour viruses with the genome of the host has been discussed before (Temin, 1966; Payne & Chubb, 1968).
The complement-fixing antigen of Reaseheath I line chickens is controlled by a dominant autosomal allele, which segregates in I line × C line hybrids in a Mendelian manner (Payne & Chubb, 1968). The cistron for the group specific, complement-fixing antigen of an integrated helper virus, ‘β’ would not itself complement RSV(O) (see above) but a linked cistron for an envelope protein might be expressed, at least after infection or transformation with RSV(O). The infectious propagation of RSVβ(O) on quail cells following multiple infection suggests, if the integrated helper hypothesis is correct, that RSV(O) infection activates the synthesis or maturation of the integrated helper ‘β’. Attempts to gain further evidence for integrated or non-integrated helper viruses of RSV(O) are in progress.

It is, perhaps, unfortunate that BRYAN strain RSV has been adopted so widely as a model of defectiveness for studies on other tumour viruses, and in textbooks (e.g. Watson, 1965; Luria & Darnell, 1967; Cohen, 1969). When Dougherty & Di Stefano (1965) reported the synthesis of virus particles by L-R cells it became apparent that the previous interpretation of the defectiveness of RSV, based on the exemplary experiments of Hanafusa and his co-workers, was itself defective. Some of the results presented here suggest that infectious RSV(O) may not, after all, be synthesized without a helper virus. The precise control of RSV synthesis in the absence of known helper viruses, regarding both host range and rate of maturation, has yet to be determined.

I am most grateful to Dr L. N. Payne and Mr R. C. Chubb for the CAM pock assays and COFAL tests and for helpful discussions about the experiments and manuscript. I am grateful to Dr R. Bassin for inoculating the hamsters and to Drs R. J. Hay and R. J. C. Harris for critically reading the manuscript. I thank Mrs Sue Ansell and Miss Maria McCrossan for their excellent technical assistance.

REFERENCES

RSV(O): Host range


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