The Complement-fixation Test for Avian Leukosis

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SUMMARY

Sera from hamsters bearing tumours induced by the Schmidt–Ruppin strain of Rous sarcoma virus (SR-RSV) contain antibodies to the avian leukosis group specific antigen and an antigen of chick origin. The ‘chick embryo antigen’ is found in chick embryos infected and uninfected with avian leukosis viruses and in chicken tumours induced by avian leukosis viruses; for example, Rous sarcoma wing web tumour and plasma from avian myeloblastosis virus infected chickens. The ‘chick embryo antigen’ has been detected in SR-RSV hamster tumour cells. Cultured chick fibroblasts do not contain the ‘chick embryo antigen’ and thus the avian-leukosis group specific antigen can be specifically detected by complement fixation using SR-RSV tumour-bearing hamster sera. This COFAL test is as sensitive as the Rubin interference test (RIF test) and unlike the RIF test will detect all known avian leukosis viruses.

INTRODUCTION

Hamster tumours induced by the Schmidt–Ruppin strain of Rous sarcoma virus (SR-RSV) contain an antigen associated with avian leukosis viruses (Huebner et al. 1964). Sera from these hamsters contain antibody that reacts with all avian leukosis viruses so far tested (Sarma, Turner & Huebner, 1964; Armstrong, Okuyan & Huebner, 1964; Vogt & Ishizaki 1965). Sarma et al. (1964) used this finding to develop a test for avian leukosis viruses called the COFAL test (Complement Fixation for Avian Leukosis). This test has the advantages over the Rubin interference test (Rubin, 1960) that it is simpler and that it will detect all such viruses, whereas the latter is subgroup specific (Vogt & Ishizaki, 1965; Vogt 1965; Hanafusa, 1965). However, embryos from chickens free from avian leukosis were found by Dougherty & Di Stefano (1966) to fix complement with sera from SR-RSV tumour-bearing hamsters, which has suggested that the COFAL test may be non-specific.

We have investigated the antigens in chick embryo cells that react with sera from tumour-bearing hamsters and found that it is possible to recognize the antigen due to avian leukosis virus. We have also compared the sensitivity of the COFAL test with the Rubin interference test to determine its suitability for the routine control of virus vaccines prepared in chick cells.

METHODS

Viruses. SR-RSV and Rous associated virus (RAV-I) were obtained from the Imperial Cancer Research Fund Laboratories, Mill Hill, London, N.W. 7. Bryan strain (T2 ct933) Rous sarcoma virus (BS-RSV) was prepared as described by Fur-
minger (1967). Avian myeloblastosis virus was propagated in day-old chicks and split by sodium dodecylsulphate using the method as described by Eckert, Rott & Schäfer (1964) for sodium laurylsulphate.

Chick embryo fibroblast cultures. Primary chick embryo cultures were prepared from 10-day-old embryos, free from avian leukosis viruses. After removing the eyes, the embryos were forced through a hypodermic syringe and the homogenate was washed once with 0.05 M-tris saline buffer pH 7.4 and once with 0.25 % trypsin in tris saline. The homogenates were trypsinized by continuously stirring with a magnetic stirrer for five min. at 22° in approximately 10 ml. of 0.25 % trypsin in tris saline. The supernatant fluid containing the released cells was filtered through gauze into calf serum to give a final concentration of 5 % calf serum. The process was repeated six times. The cells were centrifuged (500 g; 10 min.), resuspended and counted in growth medium consisting of minimum essential medium (Eagle, 1959) containing sodium bicarbonate 0.15 %, trypose phosphate broth 10 % and calf serum 5 %.

The primary cultures were grown for 3 days in Roux bottles seeded with 50 x 10⁶ cells in 125 ml. growth medium. They were then passaged by the following procedure: the cell sheets were washed with tris saline, then 20 ml. of a mixture of 0.4 % EDTA and 0.5 % trypsin in 0.005 M-phosphate buffered saline at pH 7.4 was added and decanted 10 sec. later. After a few minutes the cells were detached from the glass, and they were then resuspended in growth medium and counted. The cells were grown in medical prescription bottles seeded with 200,000 cells/ml.; 20 ml., 50 ml. and 65 ml. growth medium were used in 4 oz, 10 oz and 16 oz bottles respectively.

Focus assays. Chick fibroblast suspensions, containing 10⁶ cells in 5 ml. growth medium, were added to 60 mm. plastic Petri dishes, superinfected with serial tenfold dilutions of Rs-RSV, and incubated at 37° for 24 hr in an atmosphere of 5% CO₂ at pH 7.2. The culture fluid was decanted and the cells were overlaid with 8 ml. 0.9 % agar in growth medium. Foci were counted on day 7 and a tenfold or greater depression of Rous foci was taken as evidence of infection with avian leukemia viruses.

Production of antibody to the avian-leukosis group specific antigen. Wing web tumours were produced by subcutaneous inoculation of Sr-RSV into avian leukemia free chickens. Sr-RSV tumours were initiated in 1-day-old hamsters by inoculation of a 20 % (w/v) tumour homogenate of a wing web tumour in tris saline. The hamster tumours were serially passaged in weanling hamsters. Sera from hamsters bearing tumours (RSV-hamsters) contained antibody to the avian-leukosis group specific antigen and to 'chick embryo antigen'.

BHK 21 clone 13 hamster tumours. Tumours were produced in weanling hamsters by subcutaneous inoculation of 6 x 10⁶ BHK 21 clone 13 cells (Macpherson & Stoker, 1962).

Growth of E 50 cells. These cells are Sr-RSV hamster tumour cells grown in culture. A Sr-RSV tumour was removed from a hamster, chopped and stirred in 0.5 % trypsin in tris saline for 2 hr. The cell suspension was filtered through gauze and the cells centrifuged (500 g; 10 min.). The cells were resuspended and grown as a cell line in minimum essential medium (Eagle, 1959) containing bicarbonate 0.15 % trypose phosphate broth 10 %, lactalbumin hydrolysate 0.3 % and calf serum 5 %.

Complement fixation (CF). The method of Fulton & Dumbell (1949) was used. Tissue antigens were 20 % (w/v) homogenates in tris saline. Antigens in cultured fibroblasts were released by removing all but 0.5 ml. of the medium from a 4 oz bottle culture
and then freezing and thawing the whole culture three times. The resulting cell homogenate was used as antigen without additional treatment. Four units of antigen or antibody determined by chessboard titrations were used to measure antibody or antigen respectively.

*Gel diffusion.* The technique described by Ouchterlony (1953) was used.

**RESULTS**

Complement-fixation studies of the antigen antibody reaction between chick embryos and the sera from sr-RSV tumour-bearing hamsters

Embryos from fowls free of leukosis and fowls infected by intravenous inoculation with $10^9$ p.f.u. of Rs-RSV fixed complement with RSV-hamster serum (Table 1). Fibroblasts (always tested at approximately $6 \times 10^6$ cells in the CF test, this being the number of cells present in a well-grown 4 oz bottle of cells) obtained from these embryos gave a lower CF titre, because fewer cells were present in the test suspension than in the tissue homogenates. Only about 50% of embryo homogenates, derived from leukosis-free flocks fixed complement with sera from hamsters bearing RSV-induced tumours. The flock from which the embryos came did not possess antibody to the subgroups A and B of avian leukosis viruses and leukosis was not detected by clinical or *post-mortem* study. Also the absence of an avian leukosis virus infection in these embryos was shown by the Rubin interference test (Rubin, 1960) and the COFAL test (Table 1). The RSV-hamster sera must therefore contain antibodies to a 'chick embryo antigen' as well as to the avian-leukosis group specific antigen. In the performance of the COFAL test the fibroblasts are propagated in series and during passage the CF titre falls if the embryo homogenate initially contained the 'chick embryo antigen', which is not propagated and subsequently rises again if the embryos have been infected with avian leukosis virus (Table 1).

Complement-fixation studies of the embryo antigen in various tissues

The antigen present in chick embryo homogenate could not be removed by washing three times with tris saline. Allantoic fluid and yolk sac from positive embryos did not fix complement. In addition to RSV-hamster sera, pooled normal hamster sera and pooled sera from hamsters bearing tumours induced by the subcutaneous inoculation of BHK 21 cells were tested against a variety of antigens of chicken origin derived from birds infected and not infected with avian leukosis viruses (Table 2).
All three antisera contained antibodies to chicken liver, lung and kidney from avian-leukosis-free chickens and to BHK 21 and E 50 cells; the nature of these antibodies was not determined. The avian leukosis virus containing preparations, sr-RSV chicken wing web tumour, sr-RSV hamster tumour, avian myeloblastosis virus

![Graph](image)

**Fig. 1.** Rise in CF antibody to various antigens in sr-RSV tumour-bearing hamsters. ○—○, Chicken lung; ●—●, sr-RSV wing web tumour; □, chick embryo.

**Table 2.** Titres of chick, hamster and avian leukosis virus antigens with various hamster sera

<table>
<thead>
<tr>
<th>Antigen (20% (w/v) homogenates in tris saline)</th>
<th>Reciprocal of antigen CF titre with stated antisera*</th>
<th>Normal hamster</th>
<th>BHK tumour-bearing hamster</th>
<th>sr-RSV tumour-bearing hamster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken liver</td>
<td></td>
<td>48</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Chicken lung</td>
<td></td>
<td>8</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Chicken spleen</td>
<td></td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Chicken muscle</td>
<td></td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Chicken kidney</td>
<td></td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>sr-RSV chicken wing web tumour</td>
<td></td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>24</td>
</tr>
<tr>
<td>Chick embryo</td>
<td></td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>24</td>
</tr>
<tr>
<td>sr-RSV hamster tumour</td>
<td></td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>4</td>
</tr>
<tr>
<td>E 50 cell line</td>
<td></td>
<td>512</td>
<td>1024</td>
<td>16</td>
</tr>
<tr>
<td>BHK 21 cell line</td>
<td></td>
<td>128</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Avian myeloblastosis</td>
<td></td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>256</td>
</tr>
<tr>
<td>RAV-I infected fibroblasts</td>
<td></td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>64</td>
</tr>
<tr>
<td>Uninfected fibroblasts</td>
<td></td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
</tbody>
</table>

* Tested by complement fixation with 4 units antibody.
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(sodium deodecylsulphate split plasma from infected chickens) and RAV-I infected fibroblasts all fixed complement with the RSV-hamster serum, as did the normal chick embryo homogenate. The results indicate that the RSV-hamster antisera are the only hamster sera containing antibodies to both ‘chick embryo antigen’ and avian-leukosis group specific antigen.

These results are confirmed in Fig. 1. This shows the levels of various antibodies in hamster serum to chicken lung, chick embryo and sr-RSV wing web tumour at intervals after transplantation of sr-RSV hamster tumour cells into a weanling hamster. Antibodies to chicken lung were present throughout but those to chick embryo and sr-RSV wing web tumour each developed after 41 days, and at the same rate.

Complement-fixation studies of avian leukemia and ‘chick embryo antigen’ in various hamster sera

Sera from a number of hamsters bearing tumours induced by sr-RSV were tested by complement fixation for antibodies against avian-leukosis group specific antigen (RAV-I infected chick fibroblasts) and ‘chick embryo antigen’. The results are shown in Table 3. The antibody titres in the sera to the two antigens vary and do not bear a constant relationship to each other. Thus a number of sera shows similar titres to the two antigens whereas others give over a tenfold lower titre against ‘chick embryo antigen’. With GL tumour line 1 the antibody titre to the ‘chick embryo antigen’ has decreased with the passage level of the tumour. These results suggest that two types of antibody are present in hamster sera, one reacting with avian-leukosis group specific antigen and the other with ‘chick embryo antigen’.

Demonstration of distinct ‘chick embryo’ and avian-leukosis group specific antigen by gel diffusion

Normal hamster serum produced faint precipitin lines with the antigen it reacted with in CF tests. The titre of this serum (1/120) was not sufficient to produce strong

<table>
<thead>
<tr>
<th>Tumour line in hamsters</th>
<th>Serum code</th>
<th>Chick embryo antigen</th>
<th>Avian-leukosis group specific antigen (RAV-I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL tumour line 1. P 5</td>
<td>HP 5</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>GL tumour line 1. P 5</td>
<td>HP 6</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>GL tumour line 1. P 10</td>
<td>FB/FR</td>
<td>&lt; 2</td>
<td>120</td>
</tr>
<tr>
<td>GL tumour line 1. P 10</td>
<td>FB/FR</td>
<td>&lt; 2</td>
<td>120</td>
</tr>
<tr>
<td>GL tumour line 1. P 10</td>
<td>FB/3</td>
<td>&lt; 2</td>
<td>60</td>
</tr>
<tr>
<td>GL tumour line 1. P 10</td>
<td>FB/4</td>
<td>&lt; 2</td>
<td>60</td>
</tr>
<tr>
<td>GL tumour line 2, primary</td>
<td>EZ</td>
<td>10</td>
<td>120</td>
</tr>
<tr>
<td>NIH tumour line†</td>
<td></td>
<td>80</td>
<td>60</td>
</tr>
</tbody>
</table>

* Glaxo Schmidt-Ruppin tumour line 1, passage 3.
† Tumour received in hamsters from Dr Sarma, N.I.H., Bethesda, Md., U.S.A. The tumour was initiated by cells from the 12th tissue culture of a sr-RSV hamster tumour.
lines. An RSV-hamster serum with a complement-fixing antibody titre of 1/640 produced good precipitin lines, both with chick embryo and with avian leukosis viruses (Pl. 1). The most prominent precipitin line was common to avian myeloblastosis virus, chick embryo and sr-RSV chicken wing web tumour. This appears to be produced by a chick antigen since it is given only by chick-containing preparations. The second line was shown by avian myeloblastosis virus, sr-RSV chicken wing web tumours but not by chick embryo. This was also produced by RAV-I infected fibroblast preparations and was formed by the avian-leukosis group specific antigen, because it was only given by avian-leukosis infected preparations.

The presence of 'chick embryo antigen' in RSV-hamster tumour cells

It has been shown that antibodies to the embryo antigen are produced only in RSV-tumour bearing hamsters. The E 50 line of sr-RSV hamster tumour cells produces tumours in weanling hamsters, and sera from hamsters bearing tumours produced by the 12th passage of these cells in culture contained antibody to the 'chick embryo antigen'. The embryo antigen has been detected in E 50 cells in a gel diffusion test (Pl. 2). In the figure, pass 15 of E 50 cells and chick embryo homogenate contain a common antigen reacting with RSV-hamster serum, whereas BHK 21 cells do not contain this antigen. E 50 cells were shown to contain hamster cell sap antigens by the technique described by Furminger (1965).

![Fig. 2. Scheme of the comparative tests. The COFAL and interference results from cultures within the vertical dotted lines were compared. Control uninoculated cells were passed in parallel, challenged with bs-RSV and extracted for COFAL antigen at the same time as the inoculated culture.](image)

The sensitivity of the COFAL test and Rubin interference test for the detection of RAV-I in chick embryo cells

The comparative sensitivity of the two methods of detecting avian leukosis virus was determined in cell cultures kept in an active state of growth by passaging every third and fourth day. Secondary chick fibroblasts were grown in 16 oz bottles. On day one they were inoculated with 2.5 ml. of a 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} or 10^{-8} dilution of RAV-I in tris saline. Four bottles per dilution were inoculated and four were left as uninoculated controls. The cell cultures were passaged four times. At pass 2, 3 and 4 a 4 oz bottle was seeded for each set of cultures. They were tested for avian leukosis antigen after 4 days' growth. At passes 3, 4 and 5, Petri dishes were seeded with cells from all the
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RAV-I dilutions and the uninoculated control. These cells were superinfected with 0.2 ml. of a 10^{-8} and 10^{-4} dilutions of bs-RSV at pass 3 and with 10^{-4} and 10^{-3} dilutions at passes 4 and 5 to assess interference. The foci were counted on day 7 after the cells had been stained with 1 ml. neutral red (0.1%). A schematic representation of the test is shown in Fig. 2 and the results are given in Table 4.

The results of the focus assays were compared with those of the COFAL test on the previous pass on the assumption that the interference set up by RAV-I did not increase after challenge with bs-RSV. The CF titre of the avian-leukosis group specific antigen in the RAV-I inoculated cultures began to increase at pass two after a total of 7 days in culture and the end point was reached by pass 3 after 11 days. The CF titre of the

Table 4. Comparison of the titre of RAV-I estimated by the production of COFAL antigen and by interference with focus formation by bs-RSV

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reciprocal of CF titre</th>
<th>Average foci pass 3 cells</th>
<th>Reciprocal of CF titre</th>
<th>Average foci pass 4 cells</th>
<th>Reciprocal of CF titre</th>
<th>Average foci pass 5 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P_2 cells</td>
<td>10^{-4} dil.</td>
<td>P_3 cells</td>
<td>10^{-3} dil.</td>
<td>P_4 cells</td>
<td>10^{-4} dil.</td>
</tr>
<tr>
<td>Control</td>
<td>&lt; 4 103</td>
<td>30</td>
<td>&lt; 4 32</td>
<td>0</td>
<td>&lt; 4 64</td>
<td>0</td>
</tr>
<tr>
<td>RAV-I 10^{-4}</td>
<td>16 56</td>
<td>28</td>
<td>6 0</td>
<td>0</td>
<td>64 0</td>
<td>0</td>
</tr>
<tr>
<td>RAV-I 10^{-5}</td>
<td>4 78</td>
<td>28</td>
<td>16 0</td>
<td>0</td>
<td>48 0</td>
<td>0</td>
</tr>
<tr>
<td>RAV-I 10^{-6}</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>RAV-I 10^{-7}</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>RAV-I 10^{-8}</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Uninfected control</td>
<td>&lt; 4 89</td>
<td>51</td>
<td>&lt; 4 19</td>
<td>0</td>
<td>48 0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Too numerous to count. † End point in bold type.

The effect of active cell growth on the development of the avian-leukosis group specific antigen

Secondary cultures of chick fibroblasts in 4 oz bottles were inoculated with 0.5 ml. of a 10^{-8}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, or 10^{-8} dilution of RAV-I on day 1. Three bottles per
dilution were inoculated and three were kept as controls. One bottle of each dilution
and one control were tested for antigen on days 7, 10 and 14. The results are shown in
Table 5. By day 14 the titre of RAV-I estimated by the COFAL test was $10^4$ TCD 50/
0.5 ml. This is 1000-fold lower than the titre reached on day 11 by the interference and
COFAL test, in which cells were maintained in active growth by passing the cultures
alternatively every third and fourth day.

DISCUSSION

Avian-leukosis-virus-free chick embryos fix complement with RSV-hamster serum.
Dougherty & Di Stefano (1966) also observed this and postulated that the ‘chick
embryo antigen’ and the avian-leukosis group specific antigen were immunologically
indistinguishable. We found by complement fixation that pooled normal hamster sera
and pooled sera from hamsters bearing BHK 21 tumours do not contain antibodies to
the ‘chick embryo antigen’ or to the avian-leukosis group specific antigen. Antibodies
to these antigens are found only in RSV-hamster serum. Evidence for the existence of
two antigen antibody systems is fourfold. Two precipitin lines are detected by gel
diffusion. The ‘chick embryo antigen’ is present in tissue from avian-leukosis-free
embryos; that is, embryos from flocks free from avian leukosis on the basis of (a) absence
of antibodies to avian leukosis viruses in the flock, (b) absence of avian leukosis
viruses in the embryos by the RIF and COFAL tests, (c) absence of leukosis in the
flocks by clinical and post-mortem study. Sera from individual hamsters bearing
SR-RSV tumours have widely different ratios of titres to the two antigens in CF
tests. Passing of fibroblasts from embryos containing the ‘chick embryo antigen’
does not propagate the ‘chick embryo antigen’ whereas the avian-leukosis group specific
antigen increases in titre on passaging in avian-leukosis-virus-infected fibroblasts. The
‘chick embryo antigen’ has been detected in RSV-hamster tumour cells (E 50) by gel
diffusion, but not the avian-leukosis group specific antigen. Berman & Sarma (1965)
also found that RSV-hamster serum did not form a precipitin line with the homologous
tumour cells, but the antigen must be present in these cells for antibodies to be produced.
The ‘chick embryo antigen’ was also found in the SR-RSV chicken wing web tumours and
avian-myeloblastosis-infected plasma examined, in addition to chick embryos. Since SR-
RSV hamster tumours are initiated by inoculation of SR-RSV chicken wing web tumour
into day-old hamsters, it is not surprising that the sera from these hamsters contain anti-
obodies to the ‘chick embryo antigen’ as well as to the avian-leukosis group specific
antigen. Serial transplantation of the SR-RSV tumour cells might be expect to result in
the loss of the ‘chick embryo antigen’. This was found when the tumour cells were
passaged 10 times in vivo but not after 10 passages in vitro, in the form of the E 50 cell
line. This is probably explained by the difference in the number of cell generations
involved in 10 passes in hamsters where each pass takes about 3 months, and in tissue
culture where it takes 3 to 4 days. On the other hand one passage of chick embryo
fibroblasts is sufficient to lose the ‘chick embryo antigen’. At present the origin of the
‘chick embryo antigen’ is unknown but it may be a contaminating virus other than an
avian leukosis virus. If the ‘chick embryo antigen’ is of chick origin, physical carriage
of this antigen by hamster tumour cells must be discounted, as the antigen is lost so
rapidly from chick fibroblasts on passage. It is possible that E 50 cells are hybrids
derived from hamster and chick cells induced by Rous sarcoma virus and similar to
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those described by Harris & Watkins (1965), but the E 50 cells, unlike the hybrids, multiplied rapidly and their karyotype (kindly determined by Dr G. J. Christofinis of our laboratories) was similar to that of SV 40-induced hamster tumour cells described by Cooper & Black (1965). They appeared to contain only hamster chromosomes and were shown to be of hamster origin using the immunodiffusion technique of Furminger (1965). However, these facts do not completely exclude an hybrid.

One object of our work was to assess the value of the COFAL test for detecting avian leukemia during the manufacture of virus vaccines. The avian-leukosis group specific antigen is an internal component of the virus particle (Kelloff & Vogt, 1966) and we have shown that it is distinct from the 'chick embryo antigen'. Thus the COFAL test only measures the viral antigen when the COFAL test system does not contain the 'chick embryo antigen'. Such a system is provided by passing chick fibroblasts in culture. The COFAL test is therefore a valid test for detecting avian leukemia viruses and it is as sensitive as the Rubin interference test (RIF) test for detecting RAV-I. Similar results with other avian leukemia viruses have been obtained by Sarma et al. (1964). The titre of RAV-I was the same whether measured by interference or by production of the common avian-leukosis group specific antigen, provided that the cells were maintained in an active state of growth by regular subculture. The COFAL test is much simpler to perform and fewer subcultures are needed to reach the end point. In the comparison between the COFAL and interference tests, the focus count on the cells were taken as indicating the resistance of the cells at the previous passage. This assumes that no additional multiplication of RAV-I takes place after superinfection with RSV and this assumption may not be valid. If it is not it strengthens the argument in favour of the COFAL test because the end point with this test is reached one passage earlier. The interference test is technically exacting and failure of focus formation can easily occur. The two main causes for lack of focus formation, however, are genetically resistant cells or embryos infected with avian leukemia viruses. The COFAL test will detect chick embryo cells infected with avian leukemia virus, but in cells that are genetically resistant an avian leukemia virus in the test sample will not be detected by either the COFAL or interference test. Moreover the COFAL test has the advantage of detecting all subgroups of avian leukemia viruses (Sarma et al. 1964; Armstrong et al. 1964; Vogt & Ishizaki, 1965).

We believe, therefore, that the COFAL test is the method of choice for detecting avian leukemia viruses in viral vaccines.

The authors wish to thank Mrs J. R. Smith and Miss I. A. Armstrong for their excellent technical assistance.

REFERENCES


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EXPLANATION OF PLATES

PLATE 1

Demonstration of distinct chick embryo and avian-leukosis group specific antigen gel diffusion. Centre wells, sera from sr-RSV tumour-bearing hamster, CFT 1/640; AMV, avian myeloblastosis virus (sodium dodecylsulphate-split) CFT 1/256; EMB, chick embryo, CFT 1/64; sr-RSV, sr-RSV wing web tumour, CFT 1/256; Lu, chicken lung, CFT 1/16; Li, chicken liver, CFT 1/16; RAV-I infected chick fibroblasts, CFT 1/64.

PLATE 2

Demonstration of the embryo antigen in sr-RSV hamster tumour cells. Centre wells, sera from sr-RSV tumour-bearing hamsters, CFT 1/640; AMV, avian myeloblastosis virus (sodium dodecyl-sulphate-split) CFT 256; EMB, chick embryo, CFT 1/64; sr-RSV, sr-RSV wing web tumour, CFT 1/256; RAV-I, RAV-I infected chick fibroblasts, CFT 1/164; E 50, pass 15 E 50 cells, CFT 1/86; BHK, BHK 21 cells, CFT 1/8.