Isolation of Rubella Virus from Brain in Chronic Progressive Panencephalitis

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SUMMARY

Rubella virus was isolated from the brain of a congenitally-infected, 12-year-old child in whom progressive mental and motor deterioration became evident at age 8 and 11 years respectively. The virus was initially isolated in a co-culture of CV-1 cells with the trypsinized brain tissue; subsequently the culture of the brain tissue also showed evidence of rubella virus infection recognized by indirect fluorescent antibody technique (IFA) using anti-rubella virus antibody prepared in rabbits as intermediate serum. Both isolates interfered with infection of BSC-1 cell lines by echovirus type 11. The interfering virus was identified as rubella virus by IFA with the specific antiserum, and it is designated as the NTr strain of rubella virus. The complement fixing antibody titre to rubella virus in serum was 1:256. The spinal fluid was anticomplementary. Rubella virus haemagglutinating antibody titre (HI) in serum was 1:8196 and in the spinal fluid 1:128. The HI antibody was of the IgG class. The corresponding HI titres to rubeola virus in serum and spinal fluid were 1:8 and < 1:2 respectively.

INTRODUCTION

Two recent studies described a hitherto unreported late complication of congenital rubella (Townsend et al. 1975; Well et al. 1975) which is manifested as a chronic progressive panencephalitis. Progressive mental and motor deterioration of the three patients in the one study (Townsend et al. 1975) began at 12 to 14 years of age and in the one patient of the other study (Well et al. 1975) mental deterioration was reported at 8 years of age and motor deterioration at 11 years of age. Clinical findings in these cases included seizures, ataxia, nystagmus, myclonic jerks and dementia, findings characteristically also seen in subacute sclerosing panencephalitis (SSPE). Brain biopsies were performed on two of the patients, one at age 22, the other at age 12. We were unable to isolate virus from the 22-year-old patient despite extensive testing (Townsend et al. 1975) but were successful in isolating rubella virus from the brain biopsy tissue of the 12-year-old patient (Weil et al. 1975). This report presents our findings in the latter case.
METHODS

Culture of specimen. Cortex and subjacent white matter from the frontal lobe were finely minced and planted directly into 2 oz bottles. Similar pieces were incubated with 0.025 % trypsin in phosphate buffer solution at pH 7.5 for 15 min at room temperature. The fluid containing the dispersed cells was removed and the pieces treated once more in the same manner. The cell suspensions from the 1st and 2nd extractions were filtered through a no. 72 stainless steel wire mesh and then centrifuged at 200 g for 10 min. The sedimented cells were collected, resuspended in medium consisting of 20 % foetal bovine serum (FBS), 80 % fortified Eagles medium in Earles balanced salt solution and antibiotics (FEE), and distributed (1 × 10⁶ cells) into 2 oz bottles. These cells and explanted tissue were incubated at 35 °C in an atmosphere of 5 % CO₂ and 95 % air. Other trypsinized cell samples suspended in FEE plus 10 % FBS were mixed in a ratio of 1 to 2 with 4 continuous cell lines: human foetal diploid lung (HFDL), African green monkey kidney (CV-1), rabbit kidney (RK 13), and baby hamster kidney (BHK 21). The co-cultures were incubated in a dry incubator at 35 °C.

Viral recovery was also attempted by routine isolation procedures. Pieces of brain tissue stored at −70 °C were thawed, homogenized and inoculated into cultures of monkey kidney, human embryonic kidney, RK 13 and BSC-1 cells. The cultures were observed for c.p.e. and were checked for rubella virus by the indirect fluorescent antibody test (IFA) and by the interference technique described later.

Fluorescent antibody study. Two micron cryostat sections of the original brain material, cultures of the patient’s cells and of the co-cultures grown on 15 mm round coverslips were examined for rubella virus antigen by IFA utilizing as intermediate sera the patient’s serum and a specific anti-rubella virus serum prepared in rabbits, as previously described by Schmidt et al. (1966). Following reaction with the intermediate sera the preparations were stained with appropriate fluorescein tagged anti-gamma globulin, viz. tagged goat anti-human gamma globulin and tagged goat anti-rabbit gamma globulin respectively. The anti-gamma globulins (Antibodies Incorporated, Davis, Ca.) were labelled by standard procedures (Riggs et al. 1958). Controls included: (1) staining of brain cultures, co-cultures and brain sections with the conjugates alone; (2) staining of the cell lines used for co-cultures by IFA as described; (3) staining by IFA of known rubella virus (RV strain originally obtained from Dr J. L. Sever and Dr G. M. Schiff, National Institute for Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Md.) infected BHK 21 cultures and uninfected BHK 21 cells as positive and negative controls respectively for the staining technique.

Interference test. Specimens of cultures and brain tissue were checked for rubella virus by the interference test as follows: (1) the specimens were frozen and thawed once, inoculated to, and passed twice in BSC-1 cells. The specimens were also passed two times in RK 13 cells followed by passage to BSC-1 cells. (2) The brain culture was co-cultured with BSC-1 cells and the co-culture was serially passed four times. The BSC-1 cultures were then inoculated with 100 to 1000 TCD₅₀ of echovirus type 11 (strain Gregory) and observed for c.p.e. due to echovirus infection. Cultures of BSC-1 cells infected and non-infected with rubella virus were included in each run as controls. If interference was demonstrated, the interfering virus was confirmed as rubella virus by IFA using the specific anti-rubella virus rabbit serum (Schmidt et al. 1966).

Elution of antibody from cryostat tissue sections. Two micron sections (on glass slides) and twenty, 16 μm sections (in test tubes) of frozen brain tissue (grey and white matter)
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were treated with 0.1 M-glycine tris buffer at pH 2.4 and also at pH 7.2 for 15 min. The sections in the tubes were eluted with 0.5 ml of the buffers and those on slides with approx. 0.3 ml of buffer. Following acid treatment the pH of the eluates in the tubes were adjusted to pH 7.2 with 10 M-NaOH. Sections on glass slides were washed with phosphate buffered saline pH 7.2 rinsed briefly with water then air dried, fixed in acetone for 10 min and stained by IFA for rubella virus antigen. The tubes containing the 16 μm sections were centrifuged at about 1250 g for 5 min and the fluid removed for antibody studies.

Antibody studies. Serum and spinal fluid samples and the brain tissue eluates were assayed for rubella virus antibody by a standardized microtitre complement fixation test (CF; Lennette, 1969) and by a standardized haemagglutination inhibition test (HI) using heparin MnCl₂ to remove non-specific inhibitors (Center for Disease Control, 1970). Serum specimens were also checked by CF for antibodies to measles, varicella, cytomegalo, herpes simplex, polio types 1 to 3 and mumps viruses, by FA for antibody to vaccinia virus, by HI for antibody to measles virus, and by the metabolic inhibition neutralization test (Schmidt, 1969) for antibody to polio virus types 1 to 3. Spinal fluid was tested for antibody to vaccinia virus by IFA and to measles virus by HI.

For determination of the immunoglobulin class of the antibody to rubella virus, 0.5 ml amounts of 1:4 dilution of serum and of a 1:2 dilution of spinal fluid were centrifuged on a 10 to 40 % sucrose gradient for 18 h at 99972 g in a SW39 swinging bucket rotor. Fractions of 0.5 ml, collected from the bottom of the tube, were checked for rubella virus antibody by the HI test. The fractions were also checked for IgG and IgM by the Ouchterlony technique, using antisera specific for the heavy chains (Behring Diagnostics, Somerville, N.J.; Ouchterlony, 1968).

Immunoglobulin studies. Serum and spinal fluid and eluates were checked by immunoelectrophoresis using anti-human IgG, A and M mixture (Behring Diagnostics; Scheidegger, 1955) and by the Ouchterlony technique with antibody to the heavy chains of IgG, IgM, IgA (Behring Diagnostics). Concentration of IgG in eluates was determined by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) using LC partigen and tri-partigen plates (Behring Diagnostics).

Electron microscopy. Tissue culture cells were washed in Tyrode solution and the cell monolayer fixed for 10 min at 4 °C with 1 % glutaraldehyde. The cells were sedimented by centrifuging at 450 g and fixed an additional hour in glutaraldehyde. The pellets were then washed in 0.1 M-phosphate buffer, pH 7.2, postfixed in osmium tetroxide, rinsed in the phosphate buffer, dehydrated in graded alcohols, and embedded in epoxy resin. Thin sections were cut, stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I electron microscope.

RESULTS

Routine isolation attempts

Virus was not isolated by the usual methods.

Co-cultures

Co-cultures of BHK 21 and RK 13 cell lines with the cells from trypsinized brain tissue grew well on passage (P). They were negative by IFA for rubella virus antigen at P3 and at P2, P5 respectively. At P5 the frozen-thawed RK 13 co-culture was negative for rubella virus by the interference test. These cultures were terminated.

Co-cultures of the brain tissue cells with CV-1 and HFDL cell lines grew poorly on glass and attempts to prepare cover slip cultures at early passages were unsuccessful. After passage
in plastic flasks, coverslip cultures at P5 (approx. 2.5 months after initial co-cultivation) were successfully established and staining of the cultures by IFA for rubella virus antigen showed scattered clones of cells and single cells with bright, finely granular cytoplasmic stain, often concentrated in one small area adjacent to the nucleus. The cultures showed no staining with the conjugates only. On subsequent passages the number of fluorescing cells increased. The CV-1 co-culture could then be passed every 1 to 2 weeks while the HFDL co-culture continued to grow poorly and the cultures were eventually terminated.

At P6 the frozen and thawed CV-1 co-culture inoculated to BSC-1 cells interfered with infection by echovirus 11. The confirming IFA test indicated that the interfering virus was rubella virus. It was designated NTr strain of rubella virus because of its neurotropic properties.

The CV-1 co-culture now in the 16th passage continues to grow well, shows no obvious c.p.e. and the majority of the cells stain specifically for rubella virus in the IFA test.

Cultures of brain tissue

The explanted tissue did not grow. Two weeks after planting of cells from the trypsinized brain tissue, islands of 1 to 2 cells were attached to the vessel wall and were growing. Growth rate was extremely slow and cultures could not be passaged until 3 months later. Subsequent to the first passage, the growth rate accelerated and passages at a 1:2 dilution could be made every 5 to 7 days. The cultures are now in their 17th passage and their growth rate is slowing. In early passages the cultures morphologically resembled a mixture of epitheloid-fibroblastic cell types. No c.p.e. was seen. At present the cultured cells morphologically resemble fibroblasts.

At P3 five cells in a coverslip culture showed fine granular cytoplasmic stain specific for rubella virus antigen by IFA. At P5 approx. 1% of the total cells on the cover slip stained specifically, this number increasing to 10 to 15% by P10. The staining was as described for the CV-1 co-culture (Fig. 1). The culture did not stain with the conjugate alone. At P4 the frozen and thawed culture was checked for an interfering virus and was negative, although several specifically fluorescing cells were seen by IFA in the RK 13 culture passage. The brain culture was therefore co-cultured with BSC-1 cells and serially passed 4 times. Most of the cells in this co-culture stained for rubella viral antigen by IFA and it was refractory to infection with echovirus in the interference test. The interfering virus was identified as rubella virus by IFA. Since it was recovered from the same brain tissue as used in the CV-1 co-culture, it will also be referred to as the NTr strain of rubella virus.

Host range

Since rubella virus was not isolated in the original co-culture with RK 13 and BHK 21 cell lines, the ability of these cell lines to support growth of the virus was in question. Therefore the cell lines were co-cultivated with the cultured brain cells at P7. Cover slip cultures at P1 of the co-culture with RK 13 showed 9 clones of staining cells by IFA and co-cultures of BHK 21 checked at P2 showed approx. 100% of the cells staining for rubella virus antigen.

Cell associated and cell free virus

To determine if virus was being released from the infected brain cultures, the supernatant fluid from a culture in P9 was filtered through an HA Millipore filter and inoculated to CV-1 cells. The cell monolayer was rinsed with medium and harvested by scraping. The resuspended cells were inoculated on to CV-1 cells. After 7 days incubation over 90% of the cells in coverslip cultures inoculated with either preparation stained for rubella virus antigen.
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Fig. 1. (a) Co-culture, passage 5, of CV-1 cells and brain cells. Stained by fluorescent antibody technique using patient's serum as the intermediate reagent. (b) Culture of brain cells, passage 17. Stained by fluorescent antibody technique using anti-rubella virus serum prepared in rabbits as the intermediate reagent. Magnification: × 936.

Cryostat sections of brain tissue

No specific staining for rubella virus antigen by IFA was seen in eluted or non-eluted sections of the grey and white matter. Prior to elution the sections stained brightly with the anti-human IgG conjugate by direct FA. After elution at either pH the sections showed little, if any, reaction for IgG. On both the eluted and non-eluted sections the cells lining vessel walls stained non-specifically with both conjugates.
Table 1. Antibody titres in serum and spinal fluid

<table>
<thead>
<tr>
<th>Specimen and test*</th>
<th>Rubella</th>
<th>Rubeola</th>
<th>Vaccinia</th>
<th>Polio types</th>
<th>Others†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>256</td>
<td>&lt;8</td>
<td></td>
<td>&lt;8</td>
<td>&lt;8</td>
</tr>
<tr>
<td>HI</td>
<td>8196</td>
<td>8</td>
<td></td>
<td>(8)‡</td>
<td>(32, 64)</td>
</tr>
<tr>
<td>Spinal fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>AC</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HI</td>
<td>128</td>
<td>&lt;2</td>
<td></td>
<td>(&lt;8)‡</td>
<td>—</td>
</tr>
</tbody>
</table>

* AC, anti-complementary; CF, complement fixation; HI, haemagglutination inhibition.
† Other viral agents, varicella, cytomegalovirus, herpes simplex and mumps viruses.
‡ Titre determined by indirect fluorescent antibody technique.
§ Titre determined by neutralization (metabolic inhibition). All titres are expressed as reciprocal of dilution.

Table 2. Analyses of eluates of brain tissue*

<table>
<thead>
<tr>
<th>Antibody titre to rubella virus</th>
<th>Ouchterlony test</th>
<th>RID mg/% of IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluate</td>
<td>CF†</td>
<td>HI†</td>
</tr>
<tr>
<td>Grey matter</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>White matter</td>
<td>2</td>
<td>16</td>
</tr>
</tbody>
</table>

* CF, complement fixation; HI, haemagglutination inhibition; RID, radial immunodiffusion.
† Same values with either extraction at pH 2.4 or at pH 7.2. Twenty 16 μm thick sections eluted in 0.5 ml of buffer. Titres are expressed as reciprocals of dilution.
‡ Values at pH 7.2 and 2.4 respectively.

Table 3. Antibody class of rubella viral antibody in serum and spinal fluid

<table>
<thead>
<tr>
<th>Sucrose gradient fraction</th>
<th>Serum</th>
<th>Spinal fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HI titre*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ouchterlony</td>
<td>IgG</td>
</tr>
<tr>
<td>1</td>
<td>— †</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>32</td>
<td>±</td>
</tr>
<tr>
<td>6</td>
<td>256</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>128</td>
<td>+</td>
</tr>
</tbody>
</table>

* Titres are expressed as reciprocals of dilution.
† Initial concentration, the undiluted fraction.

Antibody studies

Table 1 summarizes virus antibody titrations. High titres to rubella virus were present in the patient’s serum and spinal fluid. The serum had low or negative CF titres to vaccinia, varicella, rubeola, cytomegalovirus, herpes simplex, mumps and polio viruses.

Antibody to rubeola and vaccinia viruses was not detected in the spinal fluid. As shown in Table 2 eluates of the tissues at acid and neutral pH contained IgG and had low antibody titres to rubella virus. The eluates from the grey matter also showed faint traces of IgA as did the spinal fluids when reacted with anti-A chain antibody in the Ouchterlony test. IgM
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Fig. 2. Extracellular rubella virus in a BHK 21 cell line after culturing with the co-culture of brain cells and CV-1 cells. Insert shows a higher magnification of a rubella virus.

was not detected in either the eluates or in the spinal fluid by either the Ouchterlony test or by immunoelectrophoresis. Antibody to rubella virus was found only in the IgG containing fractions of serum and spinal fluid separated by sucrose density sedimentation (Table 3).

Electron microscopy

Examination of the patient's cultured cells at P11 revealed rare viral particles typical of rubella virus. Virions were more readily seen in second passage co-cultures of the patient's cultured cells (P4) with BHK 21 cells, and ranged in size from 50 to 85 nm; they were located within the cytoplasm and in the extracellular spaces. Viruses budding from the cell surfaces were not observed. No virus particles were seen in the CV-1 co-culture with the original brain tissue at P5 and P6. When the same cells at P8 were co-cultivated with BHK 21 cells, and this co-culture examined at P2, rubella virus particles were observed (Fig. 2).

All cultures, in which virus particles were seen, were positive for rubella virus antigen by IFA. However, not all cultures positive by IFA revealed viral particles by electron microscopy. Low particle titre could readily account for this discrepancy.

DISCUSSION

Isolation of virus after long term culture in vitro often raises the suspicion of laboratory contamination. With respect to the present report viable rubella virus had been handled neither in the particular laboratory unit in which the work was done nor, for that matter, in any of the other laboratories on the same floor.

Successful isolation of virus in certain diseases, as in SSPE has required the special
techniques of cultivation of the affected tissue in vitro with subsequent co-cultivation or cell fusion with continuous cell lines. The inability to isolate the virus by conventional methods has been attributed to the presence of the virus in a defective form, but could also be attributable to paucity of infectious virus particles. Techniques, successfully used for rescue of defective virus particles, should also be effective in the cultivation of infectious virus present in extremely low concentrations.

Our data in this study suggest that infectious rubella virus in very low concentration, rather than defective virus, was present in the brain tissue. Thus, infectious rubella virus was recovered from the cultured brain tissue by themselves as well as from the co-cultures with CV-1 and HFDL cells. All 3 of these cultures initially grew very poorly and could not be serially passed for several months. This may have been a fortunate chance occurrence. If only a very few virus infected cells with low growth potential were present in the biopsy tissue, they could easily be lost on passage. This appears to be the case with the RK 13 and BHK 21 co-cultures which grew well and could be serially passaged but from which no virus was isolated. Later co-cultivation studies, however, showed these cells were susceptible to the virus. In the original brain culture at P3 only < 0.05% of the cells contained viral antigen, i.e. only 5 cells, of approx. 10^4 cells. At P4, a freeze-thaw preparation inoculated into the RK 13 cell line for interference studies infected a small number of these cells as determined by IFA although the infection was insufficient to interfere with infection by the test echovirus. These data suggest infectious virus in the original brain culture was present in very low concentrations, although presence of a defective virus cannot be excluded.

In this connexion Rawls & Melnick in 1966 were able to culture tissue from 11 of 68 congenitally infected infants. Ten of these cultures were infected with rubella virus and 5 of the cultures could be serially passaged. In another report Rawls, Desmyter & Melnick (1968) calculated the number of infected cells in foetal tissue to be no more than 0.1%. Woods et al. (1966) using the FA technique identified foci of infected cells in tissues of infants with congenital rubella. These authors concluded that the minute size of the infected foci and their small numbers in tissues could well explain the failure to isolate virus from fragments of organs.

Our inability to demonstrate rubella virus antigen in sections of frozen brain tissue could have been due to masking of the antigen by antibody. However, material eluted from the brain at either pH 2.4 or 7.2 contained equal amounts of immunoglobulin and of HI antibody to rubella antigen. If antigen-antibody complexes were present one would not expect such ready dissociation of the complex at pH 7.2. Further, after dissociation one would expect to find antigen in the eluted tissue and in significant amounts to account for that concentration of eluted IgG. These data suggest that the immunoglobulin had permeated through and mainly adsorbed, non-specifically, to the brain tissue, with only minimal amounts, if any, fixed specifically to antigen. Staining by IFA could be expected whether the virus was complete or defective, if it were present in large amounts. Virus was also not seen in brain tissue by electron microscopy (Weil et al. 1975). Our findings differ from those in SSPE in that measles viral antigen was demonstrated by FA in brain tissue in cases of SSPE (Connolly et al. 1967; Freeman et al. 1967) and intracellular nucleocapsids of paramyxovirus were seen by electron microscope (Bouteille et al. 1965) prior to successful isolation of the virus (Horta-Barbosa et al. 1969; Payne, Baublis & Itabashi, 1969; Barbanti-Brodano et al. 1970).

As with the RV strain of rubella virus in a previous study (Oshiro, Schmidt & Lennette, 1969) virus particles of the NTr strain were more readily seen in BHK 21 cells than in cells of other species despite the high concentration of antigen seen by FA in the latter. Unlike the RV strain, however, the NTr virus was not seen budding from the cell surface. This
Rubella virus in progressive panencephalitis may be a reflection of a low virus concentration. Alternately it may imply failure of the NTr virus to replicate at the cell surface thus affording protection to the infected cells not only against neutralizing antibody but also against the action of cytolytic antibody, and/or cytotoxic lymphocytes on cell membranes (Steele et al. 1973). Persistence of rubella virus in the presence of antibody was demonstrated in cultures established from congenitally infected infants (Rawls & Melnick, 1966) the chronically infected state being maintained by passage of the agent to daughter cells during mitosis.

If the NTr virus is not released by budding from the cell membrane and yet cell free virus can be demonstrated, release may be by cell lysis. No overt c.p.e. was seen in the cultured cells but this would not necessarily exclude a small degree of cell lysis. The co-cultures tended to have more floating cells and slower growth rate than did the uninfected control cell lines.

Microscopic examination of the brain tissue revealed numerous perivascular cuffs of lymphocytes and plasma cells in both grey and white matter (Well et al. 1975). The virus could conceivably have been carried to the brain by such cells, as may also occur with measles virus and SSPE, since both viruses have been isolated from lymphocytes (Simons & Jack, 1968; Horta-Barbosa et al. 1971). Alternatively, the virus may have remained in a latent or defective form in the brain tissue since the in utero infection and only later became reactivated. The oldest congenitally infected child from whom rubella virus has been recovered in previous reports was a 3-year-old child (Menser et al. 1967); the virus was recovered from the lens of the eye.

Our isolation of the virus from this 12-year-old child with mental disturbance for 3 years and motor symptoms for less than one year, and our failure using the same techniques, to isolate the virus from a 22-year-old man (Townsend et al. 1975) whose motor symptoms began at age 14 is similar to the experience of Horta-Barbosa et al. (1971) with SSPE in a study of 5 patients. Measles virus was isolated from the lymph nodes of two patients with early clinical symptoms but not from 3 patients in the later stages of the disease.

As in the case in SSPE with measles virus antibody (Link, Panelius & Salmi, 1973), rubella virus antibody in the present study was of the IgG class and titres were high in both serum and CSF resulting in a low serum to CSF ratio, viz. an HI serum/CSF ratio of 64/1 (the CF titre of the CSF was not determined as the fluid was anticomplementary). Similar low serum/CSF ratios were seen in two patients of Townsend et al. 1975. In the case of the 22-year-old patient the serum/CSF ratios for CF antibody and HI antibody were both 8/1 and in the second patient of that study the serum/CSF ratio for CF antibody was 32/1 and for HI antibody, 8/1. Of particular interest in the 3 cases was the specificity of the serum antibody. In the present case and in the 22-year-old the HI titres in serum and spinal fluid were significantly higher than the corresponding CF titres, whereas in the second patient reported by Townsend et al. (1975) the reverse was true. These titres may reflect the form in which the virus persists in different patients.

Another recent report on progressive encephalitis in a 14-year-old boy, in whom congenital rubella can very probably be excluded, suggests that this complication may not be limited to congenital infection (Lebron & Lyon, 1974). This patient had high titres of rubella viral antibody in the serum and CSF with a serum/CSF ratio of 10/1. No antibody to measles virus, influenza B and parainfluenza III viruses was detected in the CSF despite a fourfold rise in serum titre to influenza B during the period of observation. Onset of symptoms was at 8 years of age.

All of these studies taken together indicate that rubella virus can be added to the growing list of viral agents involved in 'slow' infections of the central nervous system.
The expert technical assistance of Miss Shirley J. Hagens, Mrs Beatrice O’Keefe and Mrs Mary Struthers is gratefully acknowledged. We also thank the Viral Isolation Unit under Mrs Helen Ho for testing the frozen material and for the interference and metabolic inhibition tests and the Serology Staff under Mrs Florence Jensen for antibody studies.

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