lar to those seen after bilateral hippocampal lesions (*21*). The continued numerical increase of granule cells in the adult suggest that their influence on total hippocampal function grows with age.

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9. We considered the total area of granule cell nuclei in each ventral slice (A_t) to be approximately
$$A_t = (\Sigma A_n/\Sigma A_s)\,(A_g)$$
where A_n is the summed areas of all granule cell nuclear profiles, A_s is the summed sample areas, and A_g is the granular layer area. Finally, V_t was the summed products of A_t for each slice and the distance D to the next slice:
$$V_t = A_{t1}D_1 + A_{t2}D_2 + \ldots A_{ti}D_i$$
where i is the last slice to contain the ventral part of the granular layer; the same procedure was applied to all slices of the dorsal part. Since the material consisted of three-dimensional slices, not two-dimensional sections, V_t was inaccurately estimated as a result of the Holmes effect [E. R. Wiebel, *Int. Rev. Cytol.* **26**, 235 (1969); H. Elias, A. Hennig, D. E. Schwartz, *Phys. Rev.* **51**, 158 (1971)], and a correction factor was applied to the data.
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Cytomegalovirus Antibody in Cerebrospinal Fluid of Schizophrenic Patients Detected by Enzyme Immunoassay

Abstract. *By means of enzyme immunoassay techniques to detect the presence of antibody to cytomegalovirus, the cerebrospinal fluid of 178 patients with schizophrenia, 17 patients with bipolar disorders, and 11 other psychiatric patients was compared with that of 79 neurological patients and 41 normal control subjects. The cerebrospinal fluid of 20 of the schizophrenic patients and 3 of the patients with bipolar disorders showed significant increases in immunoglobulin M antibody to cytomegalovirus; no difference was found in patients on or off psychotropic medications.*

Viruses have come under increasing suspicion as possible causative agents in chronic central nervous system diseases such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease. By means of an enhanced neutralization test, schizophrenic patients were found to have an increased ratio of cerebrospinal fluid (CSF) to serum antibody directed at cytomegalovirus (CMV) compared to normal controls (*1*). Cytomegalovirus has many characteristics that suggest it as a possible causative agent in schizophrenia; these characteristics include its known neurotropism, affinity for the limbic system, and potential for latency. Infection with CMV is also more prevalent in the lower than the higher social economic populations, as is schizophrenia (*2, 3*). Using enzyme immunoassay techniques, we have now studied CMV in a large group of psychiatric, neurological, and control individuals.

Samples of CSF were obtained from 178 patients with schizophrenia, 17 patients with bipolar disorders, 11 patients with other psychiatric disorders, 79 patients with neurological disorders, and 41 normal control subjects (*4*). Research Diagnostic Criteria were utilized for the diagnosis of schizophrenia and bipolar disorders (*5*). The other psychiatric patients were diagnosed as having dementia, personality disorders, and psychoneuroses. The neurological patients had a wide variety of diagnoses including demyelinating diseases, seizure disorders, back problems, and central nervous system infections. The control subjects consisted of 10 surgical patients undergoing spinal anesthesia at the National Naval Medical Center, 16 personnel at St. Elizabeths Hospital, 14 patients in an inpatient program for drug addicts at St. Elizabeths Hospital (the patients had been off drugs for 2 to 24 months), and one volunteer from the National Institutes of Health. The hospital personnel had worked on the wards for a mean of 4.6 years, and the ex–drug addicts had been inpatients for a mean of 1.1 years; these two groups therefore shared a common environment with the St. Elizabeths patients. In addition to the CSF samples from these adult control subjects, samples were also available from 38 children (ages 1 to 16 years) with suspected meningitis at Johns Hopkins University Hospital, Baltimore, Maryland; 14 of these cases were caused by bacterial pathogens, 3 by enteroviruses, and 21 had no identifiable agent. Specimens were intermixed and run under code by an investigator unaware of the diagnosis. Antibodies to CMV antigen and class-specific antibodies were detected by enzyme immunoassay techniques (*5–13*).

Since infection with CMV is more common in the lower than the higher socioeconomic classes, it was necessary to ascertain whether the study groups had received similar exposure to the virus. We therefore tested serum specimens from 60 of the schizophrenic patients and 26 of the normal adult controls for CMV antibody (*14*). No difference was found between the two groups, suggesting that exposure to this virus had been comparable.

Samples of CSF from the first 109 schizophrenic patients and 24 control

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subjects were analyzed for CMV antigen and for IgG, IgA, and IgM antibody to CMV. No differences between schizophrenics and controls were found for IgG and IgA antibodies to CMV in either the percentage of samples positive or the mean antibody titers, and no specimens contained CMV antigen. The remaining specimens were thus analyzed only for IgM antibody to CMV.

Detectable IgM antibody to CMV was found in the CSF of 20 of 178 (11 percent) patients with schizophrenia, 3 of 17 (18 percent) patients with bipolar disorders, 0 of 11 patients with other psychiatric disorders, 2 of 79 (3 percent) patients with neurological disorders, and 0 of 41 normal controls. None of the 38 children with suspected meningitis had detectable IgM antibody to CMV. Using the χ^2 and Fisher's exact tests of statistical significance, we found that the schizophrenic patients were significantly different from the control subjects ($P = .025$, Fisher's = .0297) and from the control subjects plus the neurological patients ($P = .002$, Fisher's = .001); and that the schizophrenic patients plus the patients with bipolar disorders were significantly different from the control subjects plus the neurological patients ($P = .002$, Fisher's = .001). Serum specimens were available from 19 of the 20 patients with schizophrenia and IgM antibody to CMV in their CSF. Only one of the 19 had detectable IgM antibody to CMV in the serum. The two neurological patients with IgM antibody to CMV in their CSF were a 20-year-old woman with unusual neurological symptoms diagnosed with possible multiple sclerosis, and a 50-year-old man with back problems; psychiatric histories on these patients could not be obtained and follow-up was not possible. The group of 178 schizophrenic patients included seven pairs of affected siblings, one mother-daughter combination, and a set of affected quadruplets. Only one member of all the sib pairs was positive for IgM antibody to CMV. The CSF of the single patient from Papua New Guinea was also positive for IgM antibody.

In terms of medication, there was no difference in IgM antibody to CMV in schizophrenic patients on psychotropic medications and those who had been off medications for 2 weeks or longer. Of the 38 patients off medication, 6 (16 percent) were positive for IgM antibody to CMV; of the 140 on medication, 14 (10 percent) were positive. Two of the schizophrenic patients with CSF positive for CMV antibody were first-admission patients who had never been treated with psychotropic medication at the time of their lumbar punctures. All three patients with bipolar disorders and IgM antibody to CMV had been off psychotropic drugs for 2 weeks or longer.

The schizophrenic patients with CSF positive for IgM antibody to CMV did not differ significantly from those with negative CSF on the following clinical and laboratory measurements: age at time of lumbar puncture, race, family history for schizophrenia, clinical subtype, premorbid asociality, age of first referral to a psychiatrist, years of psychosis, response to antipsychotic drugs, degree of symptomatology at time of illness, presence of soft neurological signs, CSF elevation of total protein, CSF IgG, CSF IgG as a percentage of total protein, serum IgM, and (in a subgroup of 8 positive and 42 negative patients) computerized tomography scan results. There were 11 females and 9 males in the group that was positive for IgM antibody to CMV. The mean level of IgM antibody in the CSF (as measured by enzymatic activity) of the females was significantly higher than in the CSF of the males ($P = .032$; Students t-test).

From three of the 20 schizophrenic patients with CSF positive for IgM antibody to CMV and from all of the three positive bipolar disorder patients we obtained a second CSF specimen. Five of the six second specimens were collected within 7 weeks of the originals and were again positive. The sixth specimen was from a schizophrenic patient and was collected 1 year after first specimen was obtained; the later specimen was negative.

One-third of the schizophrenic patients were tested for serum and CSF albumin, IgG, and blood-brain barrier permeability (*15*). All were normal, suggesting that there was not a nonspecific alteration in the immune system or an increased leakage across the blood-brain barrier (*1*). The fact that most of the patients with IgM antibody to CMV in their CSF did not have detectable IgM serum antibody to CMV also suggests that our findings were not due to a simple alteration in the blood-brain barrier. The possibility of cross-reactivity between CMV antigen and other viruses or of neural antigens being exposed during the course of schizophrenia could not be excluded in this study but should be investigated.

These findings are consistent with the previous report of increased CMV antibody in the CSF of 60 schizophrenic patients (*1*). When an indirect immunofluorescence method was used to study the CSF of 18 schizophrenic patients, no CMV antibodies were found (*16*). That detection of CMV antibodies may depend on the type of assay used and that the presence of such antibodies may be associated with geographic or environmental differences in patient populations should be the subject of additional investigations.

The presence of IgM antibody to CMV in the CSF suggests active infection, reactivation of latent infection, or abnormally persistent antibodies from past infection. A recent study of IgM antibodies in patients with encephalitis caused by herpesvirus-1 showed that in two of six patients IgM antibodies persisted in the CSF longer than 2 years after the onset of the infection (*17*). It is also of interest that increased IgM antibodies have been found in the CSF of a number of patients with multiple sclerosis; however, attempts to link this IgM with a specific virus have been inconclusive (*18*).

The schizophrenic patients with CSF positive for IgM antibodies to CMV in this study had been sick (continuously or intermittently) from 3 weeks to 45 years (mean 11.9 years). These patients may represent only a portion of the total number of schizophrenic patients who were positive at some point in their illness. Further studies of the relation between CMV and schizophrenia might improve our understanding of the nature of the disease.

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discarded. The samples were stored from 1 month to 8 years at −70°C prior to analysis; samples from schizophrenic and normal subjects were randomly distributed over this period whereas samples from most of the neurological patients were 6 to 8 years old. The research was done under a protocol approved by the Institutional Review Board of St. Elizabeths Hospital.
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Transformation Induced by Abelson Murine Leukemia Virus Involves Production of a Polypeptide Growth Factor

Abstract. *Rat embryo fibroblasts transformed by Abelson murine leukemia virus (MuLV) produce and release a transforming growth factor (TGF). Production of this factor is correlated with a tyrosine-specific protein kinase that is functionally active and is associated with the major Abelson MuLV gene product, P120. Transformation-defective mutants of Abelson MuLV do not transform cells, do not have their virus coded transforming gene product phosphorylated in tyrosine, and do not induce TGF production. Abelson MuLV–induced TGF morphologically transforms cells in culture, competes with ^{125}I-labeled epidermal growth factor (EGF) for binding to cell receptors, and induces phosphorylation of tyrosine acceptor sites in the 160,000-dalton EGF membrane receptor. After purification to homogeneity, Abelson virus–induced TGF migrates as a single polypeptide with an apparent size of 7400 daltons as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.*

Abelson murine leukemia virus (MuLV) is a prototype replication-defective mammalian transforming virus initially generated as a result of genetic recombination between the Moloney strain of MuLV and transformation-specific sequences of mouse cell origin (*1–4*). In the presence of an appropriate type C helper virus, Abelson MuLV transforms embryo fibroblasts in cell culture (*5*) and induces a rapid B cell lymphoid leukemia in vivo (*6*, *7*). A characteristic property of Abelson MuLV transformed embryo fibroblasts is a marked reduction in available binding sites for epidermal growth factor (EGF) (*8*). The major Abelson MuLV translational product has been identified as a 120,000-dalton polyprotein with Moloney MuLV amino terminal structural components (p15 and p12) and an acquired sequence encoded nonstructural component (*2–4*) with tyrosine-specific protein kinase activity (*9*, *10*). The involvement of this viral gene product and its associated enzymatic activity in transformation has been established through the analysis of Abelson MuLV transformation-defective (td) mutants (*11*, *12*).

In an effort to determine the significance of the reduced EGF binding observed in response to Abelson MuLV transformation (*8*), culture fluids from Abelson MuLV nonproductively transformed rat embryo fibroblasts were harvested, concentrated, extracted with acid ethanol, and subjected to analysis of molecular size by gel filtration chromatography (Fig. 1). Individual column fractions were assayed for competition with ^{125}I-labeled EGF for binding A431 cell membrane receptors and for transformation of normal rat kidney (NRK) cells as measured by ability to form progressively growing colonies in soft agar. As shown in Fig. 1, two peaks of EGF competing activity were identified; these eluted with apparent molecular sizes of approximately 10,000 daltons (fractions 75 to 85) and 20,000 daltons (fractions 45 to 55), respectively. Activities in both peaks morphologically transform cells in monolayer culture and support anchorage-independent growth of NRK cells in soft agar (Table 1). In contrast, EGF did not promote soft agar growth in this assay. Thus the activities produced and released into the culture fluids by Abelson MuLV transformed rat cells demonstrate many of the characteristics described for growth factors produced by Moloney murine sarcoma virus (MSV) transformed mouse fibroblasts (*13*, *14*), and certain human tumor cells (*15*).

Activities of Abelson MuLV transforming growth factor (TGF) are distinguished from EGF on the basis of several immunological and biochemical criteria. For instance, neither molecular size form of Abelson MuLV TGF exhibited detectable reactivity in a competition radioimmunoassay for mouse EGF (Table 1). Abelson MuLV TGF is further distinguished from EGF by its differential solvent elution profile in high-performance liquid chromatography (HPLC). Whereas mouse EGF elutes at 28.7 percent acetonitrile in 0.05 percent trifluoroacetic acid from a μBondapak C_{18} column, the 10,000-dalton Abelson MuLV TGF elutes at 19.5 percent acetonitrile concentration (Table 1); in this respect this latter factor closely resembles Moloney MSV transformed mouse cell and human tumor cell derived TGF's. After purification by HPLC of the smaller TGF of the Abelson MuLV transformed cells, a single polypeptide was obtained with an apparent molecular size of 7400 daltons, as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2A). In contrast, when analyzed in parallel, purified EGF migrated at a distinctly lower apparent molecular size (6000 daltons).

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