marrow. On the other hand, our experience with patients with acute myeloid leukaemia suggests that ECI is of restricted value as a method of treatment *per se* in this form of the disease, for without the support of chemotherapy the effect may be to increase the total leukaemic cell mass.

B. W. B. CHAN
F. G. J. HAYHOE

Department of Medicine,
University of Cambridge.

J. A. BULLIMORE

Department of Radiotherapeutics,
Addenbrooke's Hospital,
Cambridge.

Received October 28, 1968; revised January 7, 1969.

[1] Lajtha, L. G., Lewis, C. L., Oliver, R., Gunning, A. J., Sharp, A. A., and Callender, S., *Lancet*, i, 353 (1962).
[2] Oliver, R., and Shepstone, B. J., *Brit. J. Haematol.*, **10**, 181 (1964).
[3] Schiffer, L. M., Atkins, H. L., Chanana, A. D., Cronkite, E. P., Greenberg, M. L., and Stryckmans, P. A., *Blood*, **31**, 17 (1968).
[4] Hayhoe, F. G. J., Quaglino, D., and Doll, R., *The Cytology and Cytochemistry of Acute Leukaemias* (MRC Special Report No. 304, HMSO, London, 1964).
[5] Maginn, R. R., and Bullimore, J. A., *Brit. J. Radiol.*, **41**, 127 (1968).
[6] Gilbert, C. W., and Lajtha, L. G., *Phys. Med. Biol.*, **11**, 281 (1966).
[7] Gavosto, F., Maraini, G., and Pileri, A., *Nature*, **187**, 611 (1960).
[8] Mauer, A. M., Lampkin, B. C., and Saunders, E. F., *Proc. Eleventh Cong. Intern. Soc. Haematol.*, 42 (1966).

Subacute Sclerosing Panencephalitis: Isolation of Measles Virus from a Brain Biopsy

INDIRECT evidence has suggested that subacute sclerosing panencephalitis (SSPE, Dawson's encephalitis, van Bogaert's leukoencephalitis)—hardened inflammation of the brain—is associated with measles virus. This association is supported by: (1) the presence of type A inclusion bodies in brain tissue specimens[1]; (2) specific immunofluorescence with measles antibody in brain biopsies[2]; (3) extremely high measles complement fixing (CF) and haemagglutination inhibition (HI) antibody titres in the sera and spinal fluid[2]; and (4) electron microscopic evidence of paramyxovirus-like particles and nucleocapsids in brain biopsies[3]. We describe here the successful isolation of the presumed aetiological agent of SSPE.

A biopsy of a brain from a clinically, serologically and pathologically documented child with SSPE was minced, trypsinized and cultured according to standard procedures[4]. Dispersed cells were washed three times with Hanks balanced salt solution and then resuspended in Earle's minimum essential medium (EMEM) supplemented with 10 per cent heat-inactivated foetal bovine serum (FBS) to a final concentration of approximately 8×10^5 viable cells/ml. Aliquots (2 ml.) of this cell suspension were planted in culture tubes, and the growth medium was changed every 2 days until confluent monolayers developed. Outgrowth consisted of fibroblasts with a few astrocytes scattered throughout the cell sheet. Cultures were fed with maintenance medium consisting of EMEM with 2 per cent FBS and observed for a period of 14 days. No cytopathogenic effects (CPE) appeared. Supernatant fluids from these cultures were inoculated into continuous cell lines known to be susceptible to measles, and included HeLa and HEp II. These cultures did not show evidence of a transmissible CPE producing agent.

Mixed cultures were then prepared containing the brain fibroblasts combined with HeLa or HEp II cells. For this purpose the primary brain cultures were trypsinized and resuspended in EMEM, 10 per cent FBS to a concentration of approximately 3×10^5 viable cells/ml. Mixed cultures were obtained by combining one volume of brain cell suspension with two volumes of HeLa or HEp II cell suspensions containing $1{\cdot}5 \times 10^5$ cells/ml. Growth and maintenance media were the same as described previously. Monolayers were confluent within 48 h and about 90 per cent of each sheet consisted of epithelial cells of the continuous cell lines and 10 per cent brain fibroblasts. After incubation for 5 days the mixed cultures with HeLa cells showed marked CPE (syncytial and giant cell formation). Fluids from these cultures were frozen, thawed and inoculated into HeLa monolayers and the same characteristic CPE appeared on the fourth to fifth day of incubation. Haemadsorption with 0·6 per cent rhesus monkey erythrocytes[5] was positive in these cultures; control HeLa did not haemadsorb. Indirect fluorescent antibody (FA) tests[6] with inoculated HeLa cultures using paired sera from natural measles showed specific intracellular viral fluorescence; control monolayers were nonreactive. Disrupted cells and fluids from these cultures were centrifuged and the resulting supernatant haemagglutinated rhesus monkey red blood cells when tested with standard micro methods[7]; similarly prepared control fluids did not. The haemagglutinating (HA) titre was 8, and the HA activity was inhibited by serially diluted convalescent sera, but not by the acute sera[7] from three children with natural measles. In addition, distemper antisera from ferrets did not block the HA effect of the antigen, thus confirming the specificity of the HI reaction.

Secondary brain fibroblast cultures showed normal growth and structure, but the presence of intracellular measles virus was shown by FA tests using convalescent measles sera. No FA was seen with similar tests using the acute sera from the same patients. Fluid from these cultures did not yield infectious virus when inoculated into HeLa or HEp II cell lines, but suspensions obtained by freezing and thawing the cultures produced haemagglutination of rhesus erythrocytes. This HA was inhibited by convalescent but not acute sera from children with measles.

Measles virus could be re-isolated from the original brain tissue culture using the mixed culture technique with HeLa cells noted.

The work described here establishes the isolation of measles virus (or an agent extremely similar) from the brain of a patient with SSPE. Complete infectious virus was not released by cultivation of the brain cells *in vitro*, but specific immunofluorescence demonstrated the presence of incomplete, latent infection. Mixed cultures of brain fibroblasts with HeLa cells yielded complete infectious virus.

We thank Dr Albert Z. Kapikian, National Institute of Allergy and Infectious Diseases, for providing the paired human sera from patients with natural measles.

LUIZ HORTA-BARBOSA
DAVID A. FUCCILLO
JOHN L. SEVER

Section on Infectious Diseases,
National Institute of Neurological Diseases and Stroke,
National Institutes of Health,
Bethesda, Maryland 20014.

WOLFGANG ZEMAN

Department of Neuropathology,
Indiana University Medical Center,
Indianapolis, Indiana 46207.

Received December 23, 1968.

[1] Dawson, jun., J. R., *Amer. J. Pathol.*, **9**, 7 (1933).
[2] Connoly, J. H., Allen, I. V., Hurwitz, L. J., and Millar, J. H. D., *Lancet*, i, 542 (1967).
[3] Bouteille, M., Fontaine, C., Vendrenne, C., and Delarue, J., *Rev. Neurol.*, **118**, 454 (1965).
Parker, R. C., *Methods of Tissue Culture*, third ed. (P. B. Hoeber, Inc., New York, 1961).
[5] Lennette, E. H., and Schmidt, N. J., *Diagnostic Procedures for Viral and Rickettsial Diseases*, third ed. (American Public Health Association, Inc., New York, 1964).
[6] Watson, B. K., and Coons, A. H., *J. Exp. Med.*, **99**, 419 (1954).
[7] Sever, J. L., and Zeman, W., *Neurology*, **18**, 95 (1968).