those depleted substances, not only replacing the amounts lost but also producing as a function of the degree of depletion an excess of targets as well as of other intrinsic substances. Intrinsic cellular substances may be defined for these purposes as proteins, polysaccharides, and other macromolecular substances synthesized within the cell. They may be defined for these purposes as proteins, polysaccharides, and other macromolecular substances synthesized within the cell. They do not include fuel substances, amino acids, vitamins, or ions, brought to the cell from the environment.

In defying the control of growth and form of the biologic system, the mechanism appears to be some form of adaptation of high priority. In fact, since cells from earliest times must have evolved mechanisms to respond strongly to depletive stimuli; this one of increased and excessive synthesis may be quite primitive. A simple theory of biologic response to partial depletion has been announced (4), which provides a basis, although not a mechanism, for the hyperplastic response. Evidence in this paper gives some histologic support for the theory. There may be possible implication in this for the development of the cancer cell.

Besides repletion of lost constituents, a further possible biologic advantage of a cell's regenerative response might be that of increased resistance, as suggested by the finding here reported of greatly increased local resistance to the agent used. This may be accomplished by various means but a possible one could be simply the provision of an excess of cellular target to combine with the depletive agent if and when it were to invade the cell again. A basis for this idea is derived in the above theory (4), leading also to a new explanation of antibody formation.

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Measles Antigen and Syncytium Formation in Brain Cell Cultures from Subacute Sclerosing Panencephalitis (SSPE)* (33377)

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Subacute sclerosing panencephalitis (SSPE) is a progressive degenerative, neurologic disease of children that begins with mental and motor deterioration. After an interval of weeks or months, a state of plastic rigidity, interrupted by myoclonic jerks, intervenes. Coma, with signs of decortication and often of hypothalamic dysfunction, occurs during the terminal stage of this fatal illness.

Observation of cytoplasmic and nuclear inclusion bodies in cortical neurons of patients with SSPE led Dawson to propose a viral etiology for this disease (1). Evidence for an association between SSPE and measles virus has been reviewed and includes: a history of measles infection prior to the onset of symptoms; demonstration by electron microscopy of structures in the diseased brain that are morphologically similar to the nucleocapsids

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of paramyxoviruses; detection of measles, or a related antigen by immunofluorescence in the brain of patients with SSPE; and presence of exceptionally high levels of antibody against measles virus in both the serum and cerebrospinal fluid of these patients (2). Nevertheless, there has been a recurring failure to isolate or transmit an infectious agent in animals or tissue cultures inoculated with material from SSPE patients.

The present report describes the occurrence of measles antigen and syncytia formation in cells propagated *in vitro* from the brain of a patient with SSPE, and the production of syncytia in several kinds of cultured cells when exposed to these brain cells.

Materials and Methods. Tissue specimen. Open biopsy was performed by Dr. F. C. Kriss of the Department of Neurosurgery on a patient whose diagnosis of SSPE was based upon clinical and serologic evidence. Bits of cortex in Hanks' balanced salt solution (BSS) containing antibiotics were received in each author's laboratory within 20 min from the time of biopsy. Histologic confirmation of the diagnosis was made by Dr. H. Itabashi of the Department of Neurology. Detailed histopathologic and electron microscopic studies of the tissue will be reported in a separate communication.

Cell cultures. The brain tissue was finely minced and an aliquot was treated at 36° for 30 min with 10 vol of 0.25% trypsin in BSS. A three-min gravity sediment of the trypsin-treated tissue was twice more trypsinized. Cells were sedimented by low-speed centrifugation from each trypsinization and resuspended in approximately 50 vol of minimal Eagle's medium (MEM) with 10 or 30% fetal calf serum. This suspension was used to initiate stationary cultures that were incubated at 36° in tubes and bottles, some sealed and some in an atmosphere of 5% CO_2 in air. One-tenth milliliter portions of the cell-suspension were also used to inoculate tube cultures of primary human embryo-kidney, a line of green monkey kidney cells (BS-C-1), diploid human embryo-lung (WI-38), and a line of human foreskin fibroblasts sensitive to the Schwartz strain of measles virus. These cultures were maintained in MEM containing 5% fetal calf serum. Four or five pieces of minced tissue, approximately 0.5-mm cubes, per tube were grown as roller cultures using the same medium as for the trypsin-suspended cells. Two or three pieces of the mince were also inoculated into tube cultures of the different cell lines listed above. The medium on all cultures was renewed every three to five days. Subculture of brain cells was accomplished by trypsinization and seeding at one-half the original cell density.

Immunofluorescent staining. Frozen sections of brain, and coverslip cultures of brain cells, fixed in acetone for 10 min, were examined by immunofluorescent microscopy using the indirect staining technique. Antisera employed in this technique included a rabbit antiserum to herpes simplex, a hyperimmune anti-measles serum prepared in monkeys (Microbiological Associates, Inc.); paired pre- and post-measles immunization sera from children; and serum from patients with SSPE. Fluorescein conjugated goat antisera against human gamma globulin and one against rabbit gamma globulin (Microbiological Associates, Inc.) were used.

Results. Light microscopy showed an abundance of cells with intranuclear inclusions in the brain biopsy. Immunofluorescent studies showed that these inclusions and cytoplasmic material in many of the brain cells reacted specifically with measles antisera (Fig. 1), which did not stain normal brain, nor coverslip cultures of human fibroblasts infected with herpes simplex. No specific cellular staining occurred when sections of the SSPE brain were treated with only the fluorescein conjugated antiserum against human gamma globulin. Multinucleated cells were not observed in any of the sections of brain examined.

Cultures of cells from trypsin-treated brain tissue contained small fusiform cells and a lesser number of large epithelioid cells (Fig. 2). The latter had many dark, yellowish cytoplasmic granules. Multinucleated giant cells (Fig. 3) were seen in cultures of trypsinized cells five days after implantation. These giant cells became more numerous during the next week. They were always surrounded by

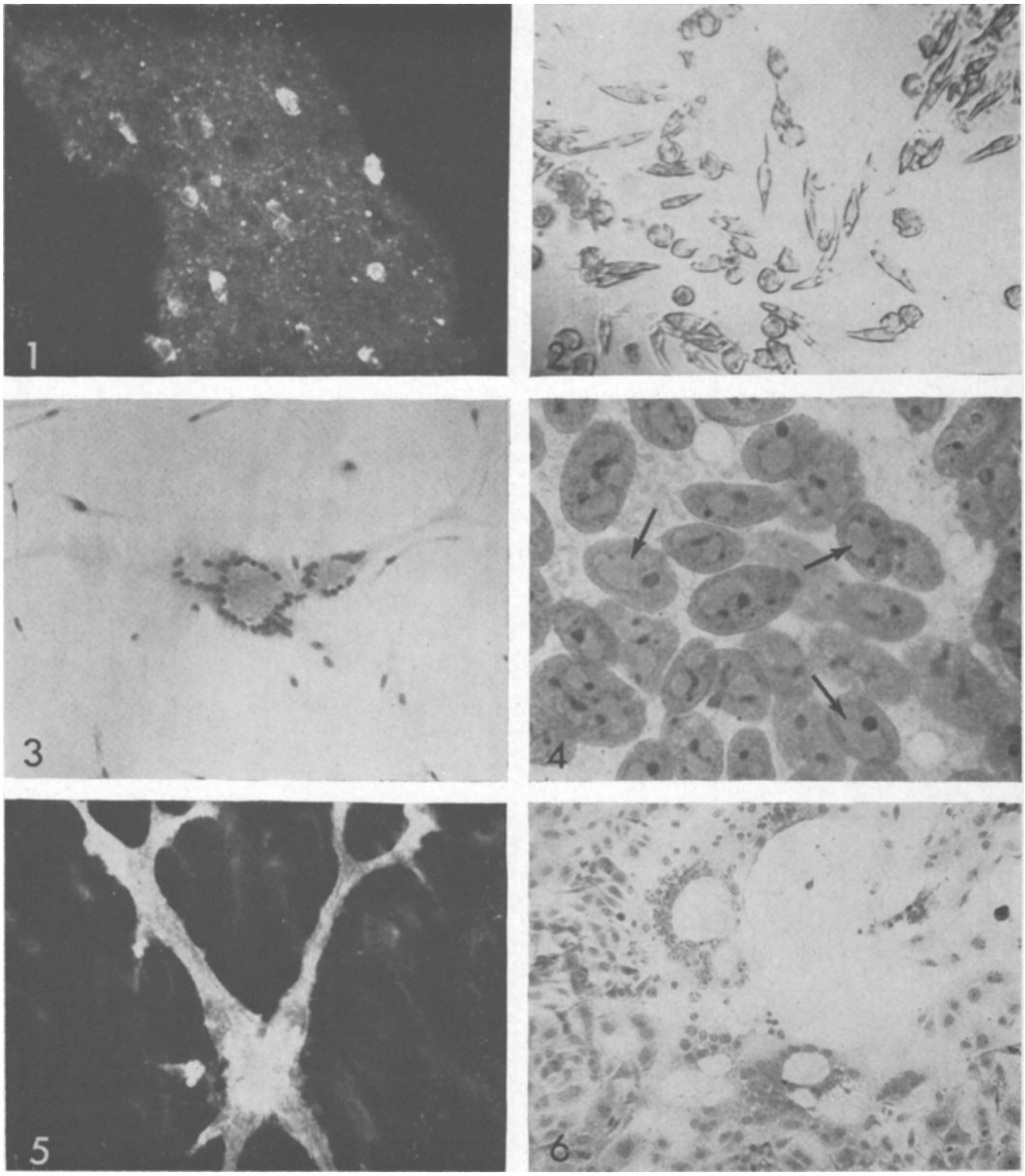

FIG. 1. Intracellular measles antigen in frozen section of a brain biopsy from the patient with SSPE. Indirect immunofluorescent antibody technique with simian antiserum to measles virus. ×220.

FIG. 2. Early cell types in primary culture of brain cells from the patient with SSPE. Unfixed preparation. ×420.

FIG. 3. Syncytium within primary culture of the SSPE brain cells. Hematoxylin-eosin; ×200.

FIG. 4. Inclusions (arrows) within nuclei of a synctium of brain cells in culture from the patient with SSPE. Hematoxylin-eosin; ×900.

FIG. 5. Giant cell with measles antigen in subculture of the SSPE brain cells. Indirect fluorescent antibody technique with simian antiserum to measles virus. ×870.

FIG. 6. Synctium in BS-C-1 cells inoculated with cells cultured from the SSPE brain. Hematoxylin-eosin; ×200.

an area free of cells, suggesting that the mononuclear cells present in the area had fused to form the giant cell. During the third week in culture, the fusiform cells proliferated rapidly and came in contact with the giant cells. Recruitment of the fusiform cells into the giant cells was then prominent after each change of culture medium. Although these syncytia enlarged, formation of new giant cells was not apparent. Subcultures of the brain cells, at first consisting almost exclusively of small fusiform, rapidly dividing cells, spontaneously developed approximately one focus of syncytial degeneration per 50,000 cells. This may not be an accurate estimate of the proportion of brain cells that had the ability to initiate syncytial formation, since plating cultured brain cells on BS-C-1 cell monolayers indicated one focus developed per 700 cells inoculated. Attempts to quantitate this phenomenon were complicated because the syncytia have been seen to undergo lysis and "heal" by ingrowth of apparently normal cells. Despite the healing process, subculture of cells from such a culture by trypsinization resulted in re-emergence of syncytia. Intranuclear inclusions (Fig. 4) have been apparent in nuclei of some syncytia in subcultures of the brain cells. Cultures established with minced brain explants did not show multinucleated giant cell formation until they were subcultured using trypsin. Subsequently, these cultures have also demonstrated syncytia formation, etc., as described for those lines initiated with trypsinized brain tissue.

Immunofluorescent microscopy of the cultured brain cells have demonstrated measles antigen in syncytia (Fig. 5) and in rare mononucleated cells through the fifth passage. Specific immunofluorescence was observed with both simian and human antisera to measles, but not with preimmunization sera lacking demonstrable measles neutralizing antibody. Immunofluorescence did not occur when herpes antiserum was employed as the intermediate serum.

Monolayer cultures of each of the simian and human cell lines noted above (see *Materials and Methods*) developed syncytia one to three weeks after inoculation with the original suspension of trypsinized brain cells, but not after inoculation with minced brain. The morphology of the syncytia suggested that they arose from fusion of cells, the majority of which were originally present in the monolayers. The numbers of syncytia increased for only a few days, but the individual areas of fusion increased in size after each change of the culture medium. Total involvement of the cultures in the cytopathic process was not seen even six weeks after inoculation.

Inoculation of monolayer cultures of BS-C-1 cells with trypsinized, cultured brain cells in fourth or fifth passage resulted in production of syncytial foci in three to four days (Fig. 6). As yet, attempts have failed to demonstrate transfer of such cytopathic activities using cell-free material from subcultures of the brain cells or from several kinds of cultures that had been inoculated with trypsin-suspended brain cells.

Discussion. The observations presented above indicate that measles virus genome is present in the cell cultures that were derived from the brain of a patient with SSPE. This is in accord with previous reports describing measles antigen in sections of the brain of patients with SSPE. The serial cultivation of brain cells, some of which contain measles antigen and produce syncytia, supports a role for measles virus infection in SSPE and provides an *in vitro* extension of the brain tissue for study of the disease. In the present study, such cells were cultured from the same brain by two geographically separate laboratories. Similar findings in both laboratories, only one of which had previously worked with measles virus, minimizes the possibility that laboratory contamination of the cultures contributed to the results.

Trypsin treatment of the original and cultured brain cells appeared to be important for syncytia formation by cells containing measles antigen. It is of interest that other investigators have successfully demonstrated measles virus, using as inocula trypsin-suspended human (3) and rodent (4, 5) cells, under circumstances where the use of tissue extracts failed. In the present study, primary outgrowth in explant cultures of brain from the patient with SSPE did not

develop syncytia. Adel *et al.* (6) also found no cytopathology or measles antigen in explant cultures of brain from three patients with SSPE.

Although syncytia were not seen in the brain biopsy from the case under study, some of the trypsin-treated cells from this biopsy were able to produce syncytia *in vitro*. In some subcultures of the brain cells, syncytia formed, lysed, and were then replaced by proliferating mononuclear cells; however, when these were subcultured using trypsin, syncytia appeared once more. These observations suggest that in the brain and in the brain cell cultures, antibody, interferon, or other inhibitors had interfered directly or indirectly with the formation of syncytia. Antiviral antibody, like that present in the brain of patients with SSPE (7), does not prevent syncytia formation by measles-infected cells *in vitro* if the infection is initiated in the absence of antibody (8). Interferon and nonspecific inhibitors of measles virus have been demonstrated in measles-infected rodent brain (5).

Factors responsible for the persistence of measles antigen and for the appearance of syncytia throughout five subcultures of the brain cells are under investigation. It is possible that these brain cells in cultures produce inhibitors of measles virus, a mutant virus for which appropriate indicator cells have not yet been used, or very small amounts of completely "normal" measles virus. Early passages of newly isolated measles virus commonly produce only low infectivity titers and show syncytial cytopathology. However, it is also possible that defective measles virus genome is responsible for the observed effects and that infected cells persisted in subculture by transfer of viral genome to daughter cells through mitosis or to neighboring cells by intimate cytoplasmic interaction. Such a system would be analogous to the HeLa cell culture described by Rustigian (9), which was persistently infected with defective measles virus.

Successful exploration of the possible role of a defective virus in the pathogenesis of SSPE requires establishment of the infection in some extrahuman system. The present report demonstrates that this requirement can be fulfilled, in at least some cases, by culturing trypsin-suspended cells from the diseased brain. Preliminary observations on cultured brain cells from two other patients have led us to suspect that the successful establishment of cell cultures, such as those described above, may be an exceptional event. Success may depend at least upon the number of cells containing measles antigen in the brain biopsy and perhaps upon the stage or duration of the patients illness.

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