abolished by a single period of 4 hours of light. Thus, this rhythm is endogenous but influenced by changes in environmental lighting. The noradrenaline rhythm, on the other hand, is like the HIOMT rhythm; it is exogenously controlled and suppressed both by continuous darkness or continuous light (*10*). Both serotonin (*11*) and noradrenaline (*12*) rhythms may be obliterated by sectioning the medial forebrain bundle.

These effects of lesions of the medial forebrain bundle on pineal monoamine rhythms and the elimination of the light-induced decrease in pineal HIOMT implies that this central neural pathway has a part in the control of certain pineal functions. Since no components of the medial forebrain bundle project caudally beyond the midbrain tegmentum, its control on distant biochemical events in the pineal must be mediated through a polysynaptic neuronal system which eventually terminates on the cervical sympathetic nerves. This is of particular interest in that the effects of lesions of the medial forebrain bundle on serotonin and noradrenaline (*7*) and on the enzyme, aromatic L-amino acid decarboxylase (*13*) in the telencephalon, are also mediated, in large part, transsynaptically. The medial forebrain bundle appears, therefore, to have a controlling influence on both peripheral and central monoaminergic neurons.

It had been suggested that brain tracts which mediate the effects of light on the pineal gland might be traced by observing the effects of their interruption on responses of HIOMT (*4*). Our studies provide evidence that one central pathway involved in the transmission of information from the retina to the pineal is the medial forebrain bundle.

JULIUS AXELROD
SOLOMON H. SNYDER*
National Institute of Mental Health, Bethesda, Maryland 20014
ALFRED HELLER
Department of Pharmacology, University of Chicago, Chicago, Illinois
ROBERT Y. MOORE
Departments of Medicine (Neurology) and Anatomy, University of Chicago

References and Notes

1. R. J. Wurtman, J. Axelrod, L. Philips, *Science* **142**, 1071 (1963); S. H. Snyder, J. Axelrod, J. E. Fischer, R. J. Wurtman, *J. Pharmacol. Exp. Therap.* **147**, 371 (1965).
2. W. B. Quay, *Gen. Comp. Endocrinol.* **1**, 3 (1963).
3. R. J. Wurtman and J. Axelrod, *Life Sci.* **5**, 665 (1966).
4. R. J. Wurtman, J. Axelrod, J. E. Fischer, *Science* **143**, 1328 (1964).
5. A. Bertler, B. Falck, C. Owman, *Acta Physiol. Scand.* **63**, suppl. 239, 1 (1964).
6. A. Heller, J. A. Harvey, R. Y. Moore, *Biochem. Pharmacol.* **11**, 859 (1962); A. Heller and R. Y. Moore, *J. Pharmacol. Exp. Therap.* **150**, 1 (1965).
7. R. Y. Moore, S. L. R. Wond, A. Heller, *Arch. Neurol.* **13**, 346 (1965); A. Heller, L. S. Seiden, R. Y. Moore, *Int. J. Neuropharmacol.* **5**, 91 (1966).
8. J. Axelrod, R. J. Wurtman, S. H. Snyder, *J. Biol. Chem.* **240**, 949 (1965).
9. S. H. Snyder, M. Zweig, J. Axelrod, J. E. Fischer, *Proc. Nat. Acad. Sci. U.S.* **53**, 301 (1964).
10. R. J. Wurtman, J. Axelrod, G. Sedvall, unpublished observations.
11. J. Axelrod, S. H. Snyder, R. Y. Moore, A. Heller, *Pharmacologist* **8**, 187 (1966).
12. R. Y. Moore, A. Heller, J. Axelrod, R. J. Wurtman, unpublished observations.
13. A. Heller, L. S. Seiden, W. Porcher, R. Y. Moore, *Science* **147**, 887 (1965); A. Heller, L. S. Seiden, W. Porcher, R. Y. Moore, *J. Neurochem.*, in press.
14. Supported in part by research grants MH-0454 and NB-05002 from NIH.
* Present address: Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Maryland.

12 August 1966

Subacute Sclerosing Leukoencephalitis: Ultrastructure of Intranuclear and Intracytoplasmic Inclusions

Abstract. *Intranuclear inclusions found in glial cells of two cases of subacute sclerosing leukoencephalitis have been examined in the electron microscope. The inclusions are composed of filamentous tubules 170 to 230 angstroms in diameter, which bear a general resemblance to the intranuclear structures observed in cells infected with measles virus. This finding provides further suggestive evidence that a virus may be involved in the pathogenesis of subacute sclerosing leukoencephalitis.*

Subacute sclerosing leukoencephalitis (SSLE) is a slowly evolving disorder of the human central nervous system. Pathological changes are prominent in the hemispheral white matter. Characteristic microscopic changes include perivascular infiltration by plasma cells and lymphocytes, hypertrophy of astrocytes, microglial proliferation, and the destruction of myelinated axons (*1*). Intranuclear and intracytoplasmic inclusion bodies similar to those seen in known viral infections are usually present at some stage of the disease (*2*). Under the light microscope, the intranuclear inclusions appear as homogeneous eosinophilic masses which occupy a central position and displace the nuclear chromatin to the periphery (*2*).

Because of these pathological features, SSLE has long been suspected to be a disease of viral etiology. Although efforts to isolate an infectious agent by inoculating SSLE material into animals and tissue cultures have been unsuccessful (*3*), a viral etiology for the disease has received recent support from the electron microscopic observation of intracytoplasmic spherical virus-like particles in the brains of patients with SSLE (*3, 4*). These particles measure 600 to 800 Å in diameter and contain a dense central osmiophilic core approximately 400 Å in diameter, surrounded by an outer envelope. Although the morphology of these particles does not permit their definite identification with one of the major groups of animal viruses, their appearance recalls that of certain enveloped, lipid-containing viruses such as arboviruses (*5*), myxoviruses (*6*), and murine and avian tumor viruses (*7*).

In an attempt to obtain more information about the development of the virus-like particles, the ultrastructure of the intranuclear inclusions in two clinically and pathologically typical cases of SSLE was examined with the electron microscope.

Tissue from frontal cortex and white matter, cerebellar white matter, and pons was obtained from brain slices (fixed with formalin), in one case, and, from a cerebral biopsy specimen, in the other case. The specimens were fixed in osmium tetroxide, dehydrated in ethanol, and embedded in Epon and Araldite. Thin sections were cut with a Porter-Blum microtome, mounted on bare copper grids, and stained with lead acetate and uranyl acetate. Hitachi HS7 and Siemens Elmiskop I electron microscopes were used in the examination.

In both cases, the intranuclear inclusions were seen in glial cells and were composed of interwoven filamentous structures that filled and replaced most of the nucleus (Fig. 1). At higher resolution, these filamentous structures consist of fine tubules 170 to 230 Å in diameter (Fig. 2). A tubular structure is suggested by a circular hollow profile in cross section (Fig. 2) and a triple density (dark-light-dark) in longitudinal section. They measure

over 4000 Å in length, but in most cases it is not possible to establish the length of individual filaments because of their wavy course out of the plane of section. A periodic pattern is suggested in longitudinal section of the tubules. Transverse striations occur at regular intervals accounting for their cross-hatched appearance. The distance between each transverse line is approximately 115 Å.

Cells which contained intranuclear filaments also often contained cytoplasmic inclusions. These were composed of dense granular, coarsely filamentous material and lacked the regular appearance of the fine slender intranuclear tubules (Fig. 3).

As previously described (*3*, *4*), spherical virus-like particles were found in the cytoplasm of hypertrophic astrocytes (Fig. 4), cells which were not seen to harbor intranuclear filamentous inclusions. Since intranuclear filamentous inclusions and cytoplasmic enveloped particles were not observed within the same cell, a relationship between the intranuclear filaments and the spherical virus-like particles could not be established.

Aggregates of virus particles such as those seen in intranuclear inclusions occurring during the development of certain DNA viruses (*8*) were not observed in the brains of these patients with SSLE. The SSLE intranuclear inclusions do, however, resemble the intranuclear inclusions found in dog kidney cell cultures infected with measles virus (*9*). The filaments seen in measles inclusion bodies measure 150 to 200 Å in diameter and over 4500 Å in length, and are, therefore, somewhat smaller in size than those seen in SSLE inclusions. Cytochemical and immunofluorescence studies of cell cultures infected with measles indicate that eosinophilic intranuclear inclusions follow the appearance of intranuclear measles antigen and nucleic acid (*10*); the inclusions are felt to be residues which form in response to infection. The SSLE intranuclear filaments are also somewhat similar to the helical internal component of the simian parainfluenza virus SV5 which accumulates in intracytoplasmic inclusions seen in

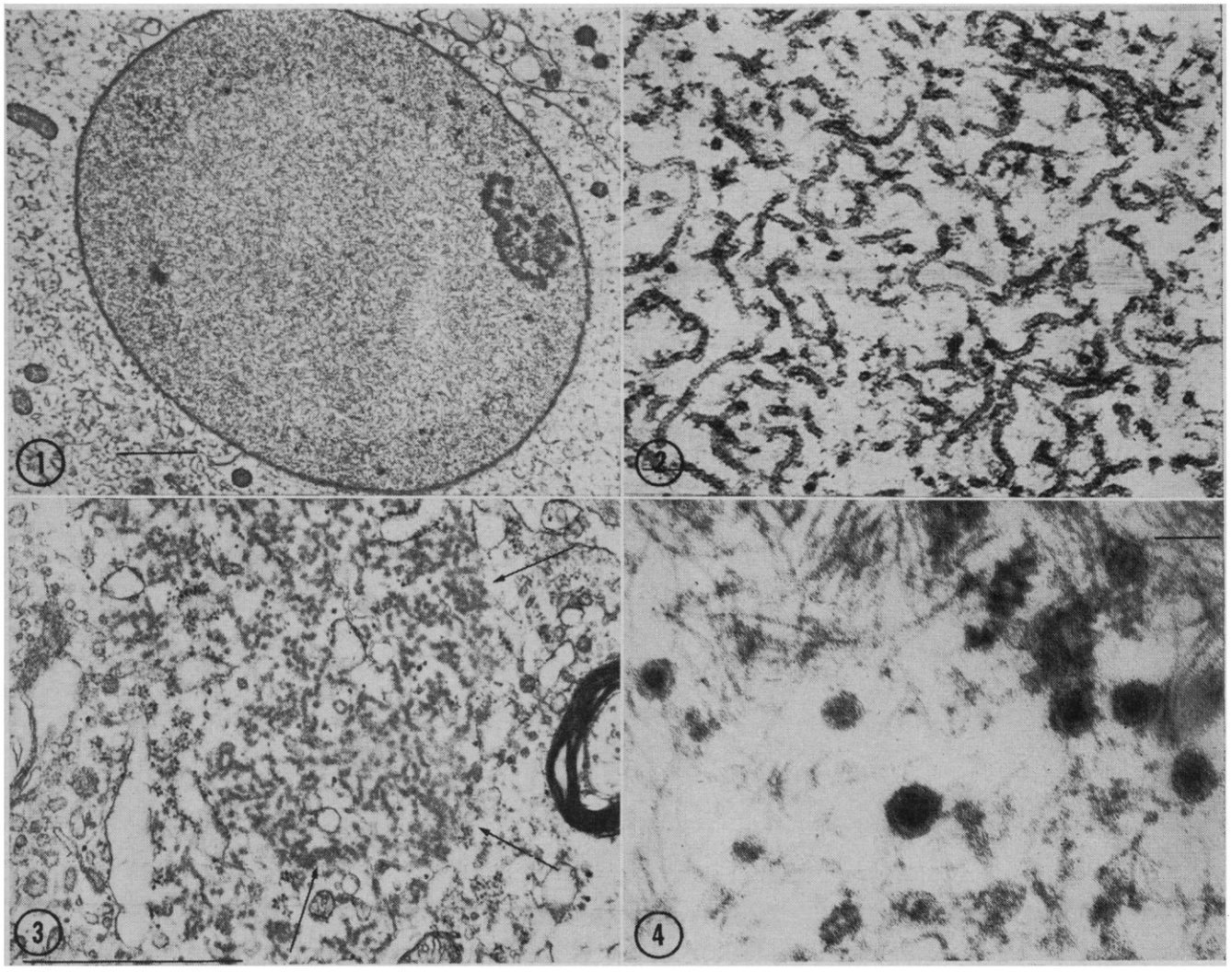

Fig. 1. Intranuclear inclusion in glial cell from frontal white matter. Fine filamentous structures occupy the center of the nucleus, filling it almost completely. The chromatin and nucleolus are displaced toward the nuclear membrane (scale indicates 1 μ). Fig. 2. High-resolution electron micrograph of the fine intranuclear tubules observed in abnormal glial cells. Cross section reveals a circular hollow profile. A transverse periodic pattern is suggested in longitudinal section (scale indicates 0.1 μ). Fig. 3. Intracytoplasmic aggregates of moderately dense, coarsely filamentous material (arrows) were present frequently in glial cells with intranuclear inclusions (scale indicates 1 μ). Fig. 4. Spherical virus-like enveloped particles, 600 to 800 Å in diameter, in the cytoplasm of hypertrophic astrocytes. The cytoplasm is rich in glial fibrils, also shown in this picture (scale indicates 0.1 μ).

baby hamster kidney cells infected with this virus (*11*). The possibility that the filamentous structures which make up the SSLE intranuclear inclusion may represent accumulations of the helical internal component of a virus should be considered.

The similarity in fine structure of SSLE intranuclear inclusions and the inclusions found in measles supports the concept that SSLE is a disease of virus etiology.

Note added in proof: Since submission of this manuscript, a report on one case of subacute encephalitis with intranuclear inclusions of identical ultrastructure has come to our attention (*12*).

ISABEL TELLEZ-NAGEL
Departments of Neurology and *Neuropathology, Albert Einstein College of Medicine, New York*
DONALD H. HARTER
Rockefeller University, New York 10021

References and Notes

1. L. Van Bogaert and J. D. de Busscher, *Rev. Neurol.* **71**, 679 (1939); L. Van Bogaert, *J. Neurol. Neurosurg. Psychiat.* **8**, 10 (1945); E. Osetowska, *Encephalitides, Proc. Symp. Neuropathol. Electroencephalogr. Biochem. Encephalitides*, L. Van Bogaert, Ed. (Elsevier, Antwerp, 1961), p. 414.
2. C. R. Schiott, *ibid.*, p. 410; W. Blackwood, W. H. McMenemey, A. Meyer, R. M. Norman, D. S. Russell, *Greenfield's Neuropathology* (Williams and Wilkins, Baltimore, 1963).
3. I. Tellez-Nagel and D. H. Harter, *J. Neuropathol. Exp. Neurol.*, in press; I. Tellez-Nagel and A. B. Johnson, in preparation.
4. N. K. Gonatas and G. M. Shy, *Nature* **208**, 1338 (1965); N. K. Gonatas, *J. Neuropathol. Exp. Neurol.* **25**, 177 (1966).
5. C. Morgan, C. Howe, H. M. Rose, *J. Exp. Med.* **113**, 219 (1961); G. H. Bergold and J. Weibel, *Virology* **17**, 554 (1962).
6. C. Morgan, R. A. Rifkind, H. M. Rose, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 57 (1962).
7. E. Harven, in *Tumors Induced by Viruses: Ultrastructural Studies*, A. J. Dalton and F. Haguenau, Eds. (Academic Press, New York, 1962), p. 183; F. Haguenau and J. W. Beard, *ibid.*, p. 1.
8. C. Morgan, S. A. Ellison, H. M. Rose, D. H. Moore, *J. Exp. Med.* **100**, 195 (1954); M. Reissig and J. L. Melnick, *ibid.* **101**, 341 (1955); S. H. Luse and M. G. Smith, *Ann. N.Y. Acad. Sci.*, **81**, 133 (1959); D. P. Block, C. Morgan, G. C. Godman, C. Howe, H. M. Rose, *J. Biophys. Biochem. Cytol.* **3**, 1 (1957); A I. Howatson and J. D Almeida, *ibid.* **7**, 753 (1960); P. Tournier, N. Granboulan, W. Bernhard, *Compt. Rend. Acad. Sci.* **253**, 2283 (1961); W. H. Gaylord and G. D. Hsiung, *J. Exp. Med.* **114**, 987 (1961); N. Granboulan, P. Tournier, R. Wicker, W. Bernhard, *J. Cell Biol.* **17**, 423 (1963).
9. J. Tawara, *Virology* **25**, 322 (1965).
10. R. Llanes-Rodas and C. Liu, *J. Immunol.* **95**, 840 (1965).
11. R. W. Compans, K. V. Holmes, S. Dales, P. W. Choppin, *Virology*, in press.
12. M. Bouteille, C. Fontaine, C. Vedrenne, J. Delarue, *Rev. Neurol.* **113**, 454 (1965).
13. We thank Dr. I. Rapin who made possible the study of the second case. Supported by NIH grants NB-02255, NB-5272-07 and NB-03356. Dr. Harter's work was supported by Special Fellowship (BT-1092) from NINDB, USPHS.

20 September 1966

Paraproteinemia and Reticulum Cell Sarcoma in an Inbred Mouse Strain

Abstract. *Mice of the inbred SJL/J strain have a high incidence of a proliferative disease affecting several cell types, including reticulum cells and plasma cells, which is frequently accompanied by γ_1 and γ_2 paraproteinemia. In only some instances can serially transplantable lines of neoplastic cells be obtained; these are reticulum cell sarcomas. Mice with transplanted reticulum cell sarcomas do not have paraproteinemia and may develop profound hypogammaglobulinemia. The disease may be viewed as an abnormal proliferation of reticulum cells which differentiate into plasma cells with consequent paraproteinemia; the subsequent emergence of transplantable reticulum cell sarcoma appears as an end stage in which this capacity to differentiate is lost.*

In 1963 E. D. Murphy described an inbred mouse strain called SJL/J which exhibits a high incidence of reticulum cell sarcoma bearing some resemblance to Hodgkin's disease (*1*). Our SJL/J colony was established in 1964 (*2*) and has shown a high incidence of the characteristic disease—84 percent at an average age of 10 months in a group of 50 virgin female mice observed for at least 14 months. Although neoplasms of similar type have been described in mice (see *3*) they are infrequent. The disease differs markedly from the common form of leukemia seen in mice of strains with a high incidence of spontaneous leukemia (for example, AKR and C58) and in mice inoculated with Gross virus. Leukemia in mice of high-incidence strains is characterized by progressive enlargement of lymphatic tissue, origin from the thymus in most cases, a rapidly fatal course, and a uniform histological picture showing proliferation of lymphoblastic cells. These leukemias have the cellular antigens associated with Gross virus (*4*) and are invariably transplantable to isogenic mice. SJL/J disease also presents with enlargement of lymphatic tissue, but it differs from leukemia of high-incidence strains in all the other respects mentioned. It does not appear to arise from the thymus, the course of the disease is very protracted, the histological picture shows both neoplastic and inflammatory features, the antigens associated with Gross virus are absent, and the disease is not readily transplantable to isogenic hosts.

A further striking difference, which we report here, is that the SJL/J disease is often associated with abnormalities in immunoglobulins. The only neoplasm of the mouse that is accompanied by comparable changes in immunoglobulins is the plasma-cell myeloma (*5*) which rarely occurs spontaneously but can be readily induced in certain strains. However, paraproteins continue to be produced by transplanted myeloma cells, whereas in the case of SJL/J disease we find that paraproteinemia is confined to primary cases and is not seen in mice with transplants.

The SJL/J disease presents with local or generalized enlargement of lymph nodes, and splenomegaly, and may occur as early as 5 months of age. (Moderate enlargement of lymph nodes may be found in even younger mice, but it is not clear how this is related to the disease.) Mice remain in good condition for long periods with progressive and often massive involvement of affected organs; the average survival time of 17 mice that developed the disease while under observation was 14 weeks. Examination post mortem at various stages of the disease shows enlargement of lymph nodes (the cervical, mediastinal, or mesenteric nodes often being grossly involved before other nodes), splenomegaly, diffuse hepatomegaly, and very prominent Peyer's patches. The number of peripheral white cells is not increased, and there may be a decrease in circulating lymphocytes. There is no anemia, even in advanced cases.

The histological findings vary greatly from mouse to mouse and even from tissue to tissue in the same mouse. The most prominent feature in early cases is proliferation of plasma cells in lymphoid organs (Fig. 1a). Later, proliferation of reticulum cells is a feature common to all cases of the disease, ranging from scattered foci to general replacement of organs. The lymphoid follicles of the spleen are progressively replaced by proliferating reticulum cells, and similar foci are found in liver, lung, thymus, and lymph nodes. Other cell types that may be found in large numbers, but with considerable variation from tissue to tissue, are eosin-