Discussion. In mouse lungs infected with the viruses of influenza and Nigg pneumonitis, a fundamental relationship between purine catabolism and virus synthesis is suggested by an increase in xanthine oxidase activity which occurs during the phase of rapid increase in virus concentration and precedes the development of maximal macroscopic and microscopic lesions. The fact that a similar change occurs after NDV which does not multiply in mouse lung(6,7) might contradict such a hypothetical relationship. On the other hand, it is conceivable that a change in purine metabolism is initiated by invasion of the host cell by viral particles whether or not subsequent multiplication of the virus takes place. Comparative studies of other types of injury to mouse lung indicate that infection with *K. pneumoniae,* and the intrapleural injection of turpentine also result in significant increases in xanthine oxidase activity. These latter findings negate the intriguing possibility that xanthine oxidase is concerned with a phase of nucleic acid metabolism intimately related to viral synthesis. The fact that a variety of microorganisms capable of producing severe and rapidly fatal pneumonias all elicit a rapid increase in xanthine oxidase activity remains of some interest. One may ask whether or not this represents a biochemical disturbance of host-cell metabolism which contributes to the over-all disease process.

Conclusion. Previous observations that xanthine oxidase increases in mouse lung concomitant with influenza virus infection have been confirmed and extended. Comparable increases in xanthine oxidase also occur in murine pneumonitis produced by Nigg pneumonitis virus and by NDV. Comparative studies with other types of infection and injury indicate that infection with *K. pneumoniae* and the intrapleural injection of turpentine also result in significant increases in xanthine oxidase activity in mouse lung thus suggesting that an increase in the level of enzyme is closely associated with injury and death to lung tissue.

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Received February 27, 1956. P.S.E.B.M., 1956, v91.

Fluorescent Antibody Studies of Demyelination in Canine Distemper.* (22293)

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The literature on central nervous system lesions in canine distemper has been adequately reviewed by Winqvist(1). Demyelination and glial cell proliferation are the outstanding changes while vascular hyperplasia and perivascular infiltrations occur less frequently. The cerebellum, pons and medulla are the principal sites of involvement. No attempt has been made to correlate the demyelination with localization of the virus. This report describes the demyelination process as it occurs in the cerebellum, and by means of the fluorescent antibody technic(2) to demonstrate the sites of viral concentration.

* This investigation was supported in part by a research grant from the National Microbiological Institute, of National Institutes of Health, Public Health Service.

† The author is indebted to Dr. J. R. Gorham for performing the *in ovo* neutralization tests, to Mrs. Lillian Frazier for the special stains, and to Mr. L. M. Hardaker for photomicrographs.

Methods. Tissues for histopathologic study were obtained at autopsy from naturally occurring cases of distemper. All the dogs had typical distemper inclusion bodies either in urinary bladder epithelium or in astrocytes of the cerebellum. Blocks of cerebellum were fixed in 10% formalin, Zenker's and ammonium bromide-formalin mixtures. Hematoxylin and eosin, Weil, Bodian protargol, pyridine silver, cresyl violet, Feulgen Mallory phosphotungstic acid, Cajal gold sublimate and oil red O. B. staining methods were employed. The fluorescent antibody technic is based on the fact that fluorescein can be conjugated with antibody molecules without loss of activity. When tissue sections containing antigen specific for the antibody solution are flooded with such fluorescein-antibody conjugate, the antibody combines with the antigen. After the excess conjugate is rinsed off, the antibody is visible under a fluorescence microscope by virtue of its fluorescent yellow-green label. *Hyperimmune canine distemper* serum was conjugated to fluorescein isocyanate according to the method of Coons and Kaplan(2). Conjugates with greater antibody titer were obtained with freshly prepared isocyanate than with isocyanate which had been previously prepared and stored. Tissues for fluorescent staining were obtained from clinical cases of distemper immediately after death. Blocks of cerebellum were placed in test tubes and quickly frozen by immersion in a bath of dry ice and alcohol. They were stored at –20°C until needed. Frozen sections were cut from these blocks at 15μ and mounted directly on slides without floating on water. Sections were dried on the slides by placing them in an incubator at 37°C for one hour. No chemical fixatives were employed. The sections were stained 30 minutes in fluorescein-antibody conjugate and then rinsed off with buffered saline, pH 9, and gently washed in fresh buffered saline for 10 minutes. Slides were wiped dry except for the section and mounted in glycerol-saline(9:1). Sections were examined in the dark with a fluorescence microscope (3). The light source was a Leitz, 15 ampere, carbon arc. Specific fluorescence appeared brilliantly yellow-green against a dark background. Fluorescent structures were identified by floating off the coverslip of the slide, counterstaining with a color dye and examining the same field under white light (3).

The *conjugate* used in this study was tested for antibody content by means of an *in ovo* neutralization test(4). The first sample had 1:630 titer and its deactivated blank, which was heated to 65°C for one hour, tested 1:250. The second sample had a titer of 1:160 and its blank, which was heated to 95°C for one hour, was negative. The difference in titers between the two samples was due to variations in quantity of virus used in the test and did not indicate a drop in antibody content in the conjugate(4). Conjugate was tested for specificity by staining smears of urinary bladder epithelium from dogs with distemper. Inclusion bodies in bladder epithelium show specific fluorescence when stained with distemper fluorescent antibody(3). *Slide controls* consisted of 1) staining brain tissues of normal 7-week-old puppies in the same manner as tissues from infected dogs, and 2) inhibiting the antigen in the tissue sections by treating with unconjugated distemper antiserum for 30 minutes prior to staining with conjugate.

Results. Weil stains of the cerebellum showed patchy areas of demyelination in the medullary white matter (Fig. 1). Early lesions appeared as empty spaces or holes in the white matter where there had been focal loss of myelin in 4 or 5 fibers. With loss of myelin, edema fluid collected in these foci, forced demyelinated axis cylinders to the periphery and compressed adjacent myelinated fibers.

When many holes formed in an area they coalesced into larger, spongy areas of demyelination (*Lückenfelder*). At the same time glial proliferation was noted. This occurred in response to disintegrated myelin for the majority of cells were microglial elements containing droplets of myelin (Fig. 2). Ameboid astrocytes were also present in the demyelinated areas. Glial scars were never observed.

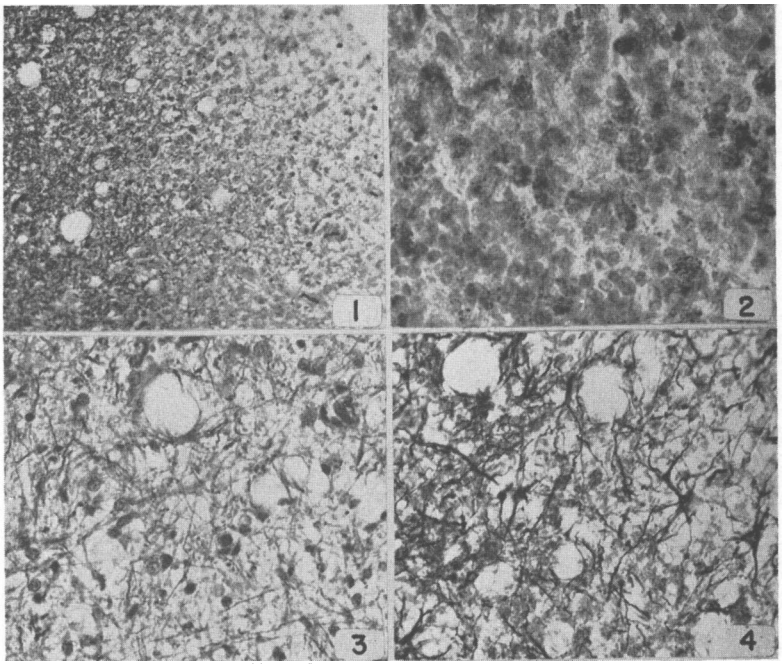

FIG. 1. Demyelination of cerebellar white matter in canine distemper. Weil stain. × 225.
FIG. 2. Microglial phagocytes filled with myelin droplets in an area of demyelination. Oil red O. B. stain. × 450.
FIG. 3. Axis cylinders in area of demyelination. Fibers show separation but no loss in continuity. Bodian protargol. × 450.
FIG. 4. Astrocytes in area of demyelination. Processes are intact although pushed apart by edema fluid. Cajal gold sublimate. × 450.

Axis cylinders were seldom involved although cylinders passing through severely demyelinated areas were stained poorly and separated by the edema fluid (Fig. 3).

The majority of fibrous and ameboid astrocytes exhibited degenerative nuclear changes. This was not limited to astrocytes in demyelinated areas, but was also found in astrocytes at a distance. Astrocytic processes were not involved (Fig. 4). The nuclei of the degenerated astrocytes appeared swollen and pale and often contained one or more acidophilic inclusion bodies (Fig. 5). Nuclear chromatin was marginated in these cells. Feulgen reactions were weakly positive in the degenerated astrocyte nuclei and negative in the inclusion bodies. Oligodendroglia showed some nuclear degeneration, but fewer cells were involved. Neurons were normal except for those in severely demyelinated areas which showed chromatolysis.

Inflammatory cells were usually absent, but in severely demyelinated areas perivascular inflammatory cells were sometimes noted. These consisted of plasma cells, lymphocytes and macrophages and were restricted to the Virchow-Robin space. A few sections exhibited proliferation of blood vessels in advanced areas of demyelination.

Sections stained with fluorescein-antibody conjugate showed specific fluorescence in the cerebellar white matter (Fig. 6). Fluorescence in the cerebellar medullary lamellae was especially remarkable because of the contrast of the non-fluorescent cortex (Fig. 7). Specific yellow-green fluorescence appeared to be localized principally in astrocyte nuclei although it was difficult to tell astrocytes from other glial cells. It was impossible to tell whether or not inclusion bodies were fluorescing. Fluorescence was not limited to cells in demyelinated areas, but involved cells throughout the white matter. Myelin sheaths, neurons and axis cylinders showed no specific fluorescence.

Control slides reacted negatively. Sections

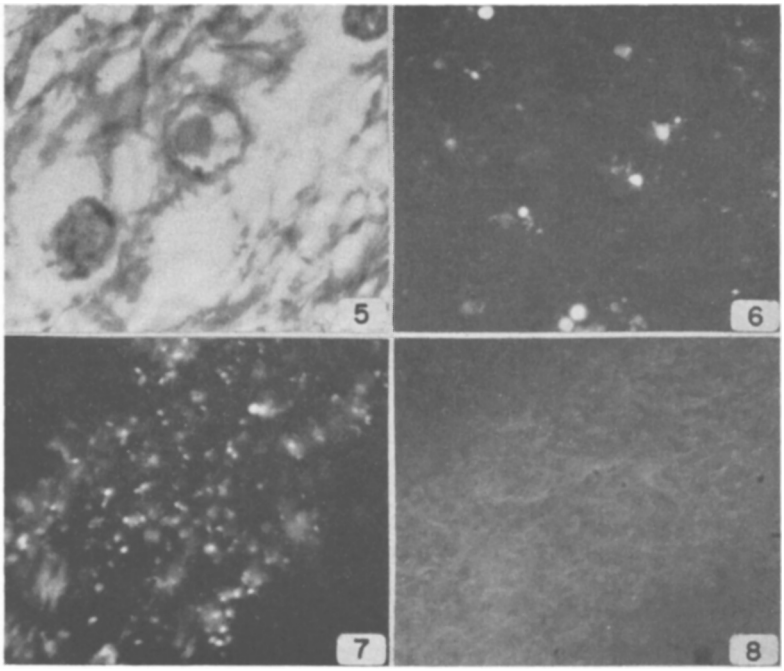

FIG. 5. In center is an inclusion body in nucleus of an astrocyte. Hematoxylin and eosin. × 600.

FIG. 6. Localization of viral antigen in glial nuclei of cerebellar white matter. Fluorescent antibody technic. × 450.

FIG. 7. Cerebellar lamella of dog with distemper showing fluorescent staining of glial cells. Note absence of fluorescence in adjacent cortex. Fluorescent antibody technic. × 400.

FIG. 8. Same area as in Fig. 7 from a normal dog. This section was treated identically. Note absence of specific fluorescence in cerebellar lamella. Fluorescent antibody technic. × 450.

from normal dogs showed no specific fluorescence (Fig. 8). In pathological specimens staining was markedly reduced in tissues treated with normal antiserum before fluorescent antibody exposure.

Discussion. Demyelination has seldom been listed among the lesions produced by viruses infecting the nervous system. Viruses have not been considered seriously as possible etiological agents of the various demyelinating maladies. There is no experimental support for the belief that the post-vaccinal type of encephalomyelitis results from direct action of the virus(5). In the case of nervous distemper, however, recent studies point to the distemper virus as the agent responsible for the demyelination and the presence of nuclear inclusions in the glial cells is evidence of the presence of the virus(6). However, the mechanism of demyelination has not been explained.

Fluorescent antibody studies of distemper indicate that neuroglial nuclei are invaded by the virus and that the astroglia are primarily involved. There is good correlation between astrocytic nuclear degeneration as observed by conventional staining methods and localization of viral antigen as demonstrated with fluorescent antibody. Demyelination in distemper does not appear to be associated directly with neuronal or axis cylinder degeneration as these structures do not show specific fluorescent staining and exhibit few degenerative changes. It appears that demyelination in this disease is associated with viral invasion and degeneration of glia, especially astroglia although this relationship cannot be explained. Other than the possible association of oligodendroglia with the production and maintenance of myelin(7), no other relationship between glial cells and myelination is known.

When sufficient myelin is broken down, microglial phagocytes invade the area and

ameboid astrocytes are evident. Perivascular inflammatory reaction is not a constant finding in this disease, but when it occurs it is probably a secondary reaction in response to disintegration of myelin(8).

Summary. Fluorescent antibody studies show that the virus of canine distemper is localized in astrocytic and possibly other glial nuclei. The astrocytes exhibit degenerative changes consistent with viral invasion. Astrocytic involvement appears to be followed by demyelination although this causative relationship cannot be explained.

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Received January 30, 1956. P.S.E.B.M., 1956, v91.

Monolayer Cultures of Trypsinized Monkey Kidney Cells in Synthetic Medium. Application to Poliovirus Synthesis.* (22294)

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The outgrowth of monkey kidney cells from suspensions of trypsinized tissue has always been found to require substantial amounts of serum in the medium. The necessity for adding serum has precluded many kinds of studies and has restricted the kind of cultures available for study. A simple synthetic medium is described which, in the absence of any added protein, supports outgrowth of monkey kidney cell suspensions. After outgrowth in this medium, the cells support synthesis of poliovirus and may be used for physiological studies under defined conditions in the absence of serum.

Methods. Cell suspensions. Trypsinization of kidneys taken from rhesus monkeys was done by a modification(1) of the Youngner procedure(2). The cells were harvested from the trypsin fluid by centrifugation at 200 rpm for 30 minutes, resuspended in 500 x their volume of Basic Salt #2 without bicarbonate (as described in Table I), and recentrifuged. The washed cells, resuspended in 100-200 ml of synthetic medium, were filtered through 2 layers of cheese cloth. A count of those cells showing both nuclei and attached cytoplasm was made by staining with 0.1% crystal violet-0.1 M citric acid, and the suspensions were diluted to 350,000 cells per ml. Amounts of 0.5 ml were seeded into 16 x 150 mm test tubes, 7 ml into 2 oz and 12 ml into 4 oz prescription bottles, and 80-100 ml into Roux flasks. These amounts are also in the optimal range for cells suspended in the medium which contains serum (1). In experiments where outgrowth in the synthetic medium was to be compared to outgrowth in a serum-containing medium, the suspension in PBS was divided into 2 portions and the second half after recentrifuging was diluted into the serum-containing medium. The serum-containing, "complete" medium (called "M") used for comparison was the 0.5% lactalbumin hydrolysate-2% calf serum medium in Hanks' salt solution which has been used extensively in this laboratory(3).

Virus titrations. These were made by the

* This investigation was aided by grant from National Foundation for Infantile Paralysis and by research contract with Division of Biologics Standards of National Institutes of Health, Public Health Service.