Table 1. Relative numbers of exposures exhibiting ripple marks.

Condition	No. of photos	Total No. of photos (%)
All rippled	138	47.5
Rippled and nonrippled	71	24.5
All nonrippled	81	28
Total	290	100

information about the nature of the current. One possibility is that we sampled one, southbound, phase of an oscillating tidal current; however, the well-developed, very long crested ripples suggest a more uniform flow regime (*3*).

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23 November 1962

Cerebral White Matter: Selective Spread of Pneumococcal Polysaccharides

Abstract. *Pneumococcal polysaccharides were implanted in rat brain and their distribution was studied by immunofluorescence. The polysaccharides spread selectively in white matter, and frequently extended from anterior to posterior poles. The results suggest that selective localization of experimental and natural leukoencephalopathies may be related to an innate property of white matter that permits or facilitates spread of noxious agents.*

Demyelinating diseases and cerebral edema are characterized by selective localization of lesions in white matter. Recently, three experimental lesions (leukoencephalopathies) localized in the white matter of rat brain have been described: edema that follows implants of certain inorganic or organic chemicals; edema and leukocytic infiltration that follows implants of purified protein derivative of tubercle bacilli, or bacterial endotoxin; vacuolation, with little edema or leukocytic reaction, which results from implants of capsular polysaccharides from pneumococcus or cryptococcus (*1*). The common pattern of distribution of these histologically different lesions suggests that a mechanism is present in white matter which permits or facilitates the spread of exogenous substances. Support for this hypothesis is presented in this report.

Pneumococcal polysaccharides of types 2 or 3, mixed with an equal weight of graphite, were implanted as solid pellets in the anterior end of the right cerebral white matter (callosal radiation) of the rat brain (*1*). At the appropriate time the rats were exsanguinated and their brains were removed and fixed in Rossman's fluid (*2*) or acetate-buffered 10 percent Formalin. Horizontal slices were embedded in paraffin. Sections were deparaffinized with xylene, and passed through 100, 95 and 70 percent ethanol into saline. Preparations of frozen sections mounted on slides from an alcohol bath, gave results comparable to those obtained with paraffin-embedded tissue. Slides were stained for 30 minutes at room temperature with antipneumococcal serum conjugated with fluorescein isothiocyanate (Sylvana), washed in saline for 10 minutes, and mounted in buffered glycerol (*2*). Observations were made with ultraviolet light and dark-field illumination on Leitz and on Reichert fluorescence microscopes.

Brilliant green fluorescence was observed in the sections from the brains implanted with type 2 polysaccharide when they were stained with conjugated type 2 antiserum (Fig. 1) but not with conjugated type 3 antiserum. Sections of brains implanted with type 3 polysaccharide showed fluorescence after staining with type 3 conjugated antiserum but not with type 2 conjugated antiserum. This type specificity of fluorescence was further substantiated. If the antibodies from the type 2 conjugated antiserum were removed by specific precipitation with type 2 polysaccharide, the residual antiserum no longer caused the staining of sections of brains implanted with the same or a different preparation of type 2 polysaccharide. If type 3 polysaccharide was added to type 2 conjugated antiserum there was no precipitation and no alteration of staining. Similarly, fluorescent staining of sections of brains implanted with type 3 polysaccharide by type 3 conjugated antiserum was prevented if the antiserum was specifically precipitated with type 3 polysaccharide but was unaffected by addition of the type 2 polysaccharide. Blocking the reaction sites with type specific but unconjugated antiserum for 30 minutes at room temperature before the conjugated antiserum was applied give slight but definite reduction of fluorescence. Four consecutive pretreatments resulted in much greater reduction of fluorescence. Neither one nor four pretreatments with unconjugated antiserum of a noncorresponding type reduced the subsequent fluorescent staining with conjugated antiserum of type corresponding to the implant. Only mild autofluorescence was observed in sections of brains with implants of unrelated materials such as silver nitrate and purified protein derivative.

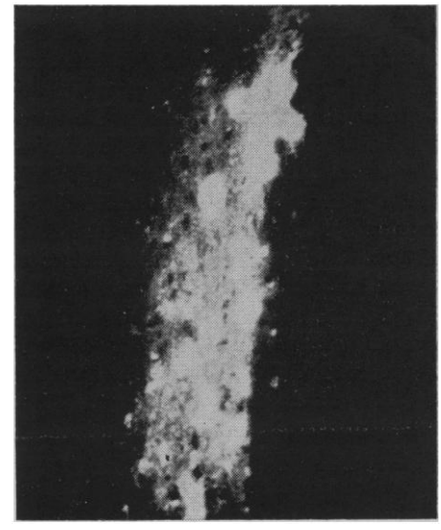

Fig. 1. Horizontal section of posterior portion of rat brain stained with fluorescein-labeled type-specific antipneumococcal serum. There is bright fluorescence in the white matter (callosal radiation), but not in the cerebral cortex (left) or corpus striatum (right), indicating pneumococcal polysaccharides spread selectively in white matter (× 125).

Fluorescence was distributed selectively in the white matter, and corresponded to the distribution of the vacuolar lesions (*1*). Fluorescence in white matter adjacent to the implant was detected 6 hours after implantation; at 12 hours fluorescence extended along the callosal radiation, and at 24 to 48 hours the entire callosal radiation was fluorescent (Fig. 1). The fluorescence reaction was reduced 1 week after implantation; preparations of brain made 2 and 3 weeks after im-

plantation showed only a few fluorescent phagocytes. Fluorescence was observed during the peak period after implantation in the corpus callosum along its border with the septum, in the midline of the septum, along the margins of the internal capsule, occasionally in anterior commissure, lateral olfactory tract, contralateral callosal radiation, and to a minor degree in white-matter bundles of the corpus striatum. In all these respects, fluorescence was parallel and coextensive with vacuolar lesions detected by ordinary microscopy. Part of the fluorescence in white matter came from phagocytes, but most of it appeared as fine lines, straight and wavy, and circlets which often outlined the vacuolar lesions. Fluorescence in gray matter came from phagocytes and was limited to the corpus striatum and the cortex of the frontal pole. Leptomeninges on the side of the implant showed bright fluorescence. The implant itself showed fluorescence of variable intensity; often it was weak or absent.

The fluorescence technique demonstrated that the polysaccharide spread into many areas. Spread into certain areas was not anticipated by ordinary microscopy. There was mild fluorescence in additional tracts of white matter (fornix, stria medullaris). Bright lines of fluorescence appeared under the ventricular ependyma; this is of interest because plaques of multiple sclerosis are seen frequently in the periventricular location.

Previous workers have demonstrated that prussian blue reagents (*3*) and radioactive isotopes (*4*) spread along white-matter tracts after injection into white matter but do not spread after injection into gray matter (*3*). The present investigation has shown a direct correlation and probably a causal relationship between the spread of foreign material (polysaccharide) along white matter tracts and a specific lesion of white matter, a leukoencephalopathy. If the results can be generalized to cover other experimental leukoencephalopathies (*1*), different exogenous materials (or mediators liberated from tissue) could spread in white matter and produce different histologic types of reaction. The type of lesion depends on the nature of the toxic agent, but the distribution of the lesion is based on an intrinsic property of white matter that allows or facilitates the spreading of noxious agents. These considerations may be pertinent to the pathogenesis of disorders with selective localization in white matter, particularly the cerebral edema that results from focal lesions (brain abscess or tumor) and perhaps to other forms of cerebral edema and demyelinating diseases (*5*).

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Amoeba proteus: Studying the Contractile Vacuole by Micropuncture

Abstract. *Direct measurements of the freezing point depression of the protoplasm and of the fluid from the contractile vacuole of fresh-water amoebae showed that the fluid in the vacuole is distinctly hypoosmotic to the protoplasm. Fourteen samples of protoplasm from amoebae, placed in a medium with a milliosmolality of 7, had an average osmolality of 101 milliosmoles with a range of 73 to 116. Eleven samples of vacuolar fluid had an average osmolality of 32 milliosmoles, with a range of 24 to 38. It is suggested that the fluid may be isoosmotic to the protoplasm when secreted and that salt is subsequently reabsorbed, leaving the vacuolar fluid hypoosmotic to the protoplasm.*

It is generally agreed that the contractile vacuoles of fresh-water protozoans serve an osmoregulatory function. Several investigators have shown that the rate of output varies inversely with the salinity of the medium and that the vacuoles cease to function in higher salinities (*1*). Indirect measurements of the osmotic concentration of the protoplasm (*2*) have shown that the protoplasm with an osmolality of 90 milliosmoles (90 mosm), normally is considerably hyperosmotic to the medium at 7 mosm. It is assumed that water entering the amoeba through diffusion is bailed out by the vacuole. Nothing, however, is known about the mechanism by which this is accomplished. The osmotic concentration of the vacuolar fluid had never been determined and it was not known if it was hypoosmotic or isoosmotic to the protoplasm.

We have measured the osmotic concentration of samples of vacuolar fluid and of protoplasm obtained from *Amoeba proteus* by micropuncture. The amoeba was held in place in a drop of fluid under a thin collodion membrane, which was prepared by placing a drop of a solution of 1 part amylacetate to 1 part collodion flexible on water (*3*). A polyethylene loop was then placed on the membrane, the excess membrane was cut away, and the loop and membrane were placed on a prepared slide containing one drop from the amoeba culture. The slide was observed under a stereomicroscope (magnification × 32) while water was withdrawn from beneath the membrane until the amoebae were pressed against the slide by the weight of the membrane. An amoeba with a large surface vacuole was then centered in the field of vision and the magnification was changed to × 125.

A prepared pipette (hand-drawn, quartz tube, tip diameter 2 to 4 μ, shaft diameter 30 to 50 μ, previously filled with oil) was centered in the field of vision above the amoeba to be punctured. The pipette was lowered by "coarse" movement of the micromanipulator to just above the surface of the membrane where it was centered over the vacuole. It was then lowered into the vacuole by "fine" movement and the sample was withdrawn only if the pipette was centered in the vacuole and the tip was visible there.

Most, but not all, of the fluid was withdrawn by suction with a syringe attached to the pipette by polyethylene tubing. The size of the sample was approximately 1×10^{-4} μl. The pipette was left in the remains of the vacuole while two or three drops of oil were placed over the membrane. The pipette was then withdrawn up into the oil and oil was pulled into it until the sample of fluid was located in the upper portion of the pipette shaft.

The freezing point depression was determined in a Ramsay osmometer (*4*). Each freezing point was determined three times. The same procedure was used for protoplasm samples except that a larger tip (diameter 4 to 6 μ) was used so that it would not become plugged with protoplasmic granules. Medium samples were obtained with the same procedure used for the other samples. They were usually collected in the immediate vicinity of the amoeba just after the puncture.