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.

Table 1. Osmolality of protoplasm and vacuolar fluid in milliosmoles.

Vacuole	Medium	Protoplasm	Medium
31	5	109	3
33		116	
32	10	109	
32	2	110	9
24	1	116	
27	4	101	
38		94	10
26		87	10
38		106	
35	10	79	
36	8	92	
		88	
	Averages		
32	6	101	8

The osmolality of the contents of mature contractile vacuoles and medium surrounding the amoeba at the time of the formation of the vacuole are given in Table 1. The average concentration of the vacuolar fluid was 32 mosm and that of the medium was 6 mosm. The osmolality of protoplasm samples is also given in Table 1. The average concentration of protoplasm was 101 mosm. These samples were not obtained from amoebae which had already been punctured because of the possible changes that might have taken place in the protoplasm following the first puncture. In two cases only, two punctures were performed on the same amoeba. In one the vacuolar fluid was 36, the protoplasm 92, and the medium 8 mosm. In the other food vacuole and protoplasm samples were obtained, the concentrations being 91 and 88 mosm, respectively.

The finding that the vacuolar fluid is distinctly hypoosmotic to the protoplasm is in agreement with the hypothesis that the vacuole serves in osmoregulation. Until a few years ago it was believed that water was secreted actively into the vacuole (*5*). If this were the case the vacuolar fluid would be hypoosmotic to the protoplasm, as we have found it to be. Active water transport, however, is highly improbable on thermodynamic grounds (*6*) as well as from a comparative physiological point of view (*7*). Another possible mechanism for the formation of the vacuolar fluid was that a solute, possibly a nitrogenous waste product, was secreted into the vacuole with the water following passively. If this were the case, however, we would expect the fluid in the vacuole to be isoosmotic to the protoplasm, which is in disagreement with our findings. A third possible mechanism is that the formation of the fluid takes place in two steps: (i) solute is secreted with water following passively, and (ii) the solute is reabsorbed leaving a dilute solution behind. If this latter explanation is correct, it is possible that the secretion of the fluid takes place in the small vesicles surrounding the vacuole and that reabsorption of the solute takes place in the vacuole itself. According to Mercer (*8*) the mature vacuole is surrounded by numerous mitochondria which are not yet present in the small growing vacuole. A vast number of tiny vacuoles or vesicles are found between the layer of mitochondria and the membrane. These vacuoles presumably burst into the main vacuole.

It has been shown by Dunham and Child (*9*) that in *Tetrahymena* sodium is extruded against a chemical concentration gradient while potassium is retained inside the cell against a gradient. The intracellular chloride concentration is much lower than the cation concentration. In dilute solutions both cations, particularly K, are retained in higher concentrations than in the medium. These authors suggest that they are retained by a system of internal binding sites with a saturation level.

The membrane of the contractile vacuole is, according to Mercer (*8*), identical in structure to the plasma membrane. If they also function alike, we might expect the vacuolar membrane to reabsorb K and extrude Na. Consequently, when these possibilities are investigated, it is most likely that we will find a lower K concentration and a higher Na concentration in the vacuole than in the protoplasm (*10*).

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Polygonal Fracture and Fold Systems in the Salt Crust, Great Salt Lake Desert, Utah

Abstract. *Small folds and fractures (thrusts) up to 50 feet apart that have produced polygonal patterns in the Bonneville salt crust, western Utah, are believed to be caused by the annual expansion of the salt crust due to the growth of salt crystals within the salt layer plus the effect of increased summer temperature. It is suggested that these strain systems are caused by positive (compressional) isotropic planar stresses developed within the salt layers of the salt crust.*

Periodic evaporation of a veneer of surficial saline water and seasonal capillary upflow and evaporation of water have caused precipitation of a crust of mineral salts, chiefly sodium chloride, over the well-known Bonneville Salt Flat racing course near Wendover, Utah. The surficial salt crust developed annually (¼ to 1 inch) and the upper layer of the perennial salt unit (2 inches) cover an area of more than 200 square miles. They overlie the main stratified salt body which ranges up to several feet in thickness (10 to 25 inches on the racing course) and which overlies unconsolidated, saline-water-saturated, thin-bedded clays and granular gypsum deposits.

Precipitation and growth of salt crystals in the upper perennial salt layer and in the surficial annual salt crust plus the effect of increased summer temperature cause essentially planar isotropic positive (compressional) stresses to develop within the tabular shaped salt units. This stress has caused the upper 2-inch layer of perennial salt to fracture (thrust) polygonally, and, over a period of time, to develop the reticulate or polygonal strain systems illustrated in Fig. 1. Polygonal fold systems develop most frequently in the annual thin (¼ to 1 inch) salt crust (Fig. 2).

The development of the polygonal strain systems does not take place simultaneously. The fractures are usually initiated at specific points on the salt layer and grow laterally at about 120 degrees from the points of inception. The points of initial strain development are believed to be determined by points of weakness in the salt layer, and they tend to be equally spaced over the salt layers. This spacing, it is suggested, is largely controlled or determined by points or areas of relative weakness within the salt layer, which fall in or near the zones where