stress is that effective steady-state sieving can occur only when a removal mechanism coexists with the rejection mechanism. Thus no effective sieving can occur within the body of a real membrane unless some removal mechanism for the rejected solute is provided; otherwise solute would continue to accumulate within the membrane, which is impossible at steady state. In the case of ultrafiltration the removal mechanism usually consists of stirring or rapid flow parallel to the upstream face of the membrane, so that effective sieving occurs only at this interface where both a means of sieving and a removal mechanism coexist. It is commonly accepted that an ultrafiltration membrane will cease to sieve effectively in the absence of stirring because of what is termed concentration polarization (*4*).

Finally, on the basis of the foregoing considerations we can conclude that separation processes such as those operative in chromatography, which depend on differences of interaction between each species and the matrix throughout the column, will be effective only in the transient mode and will become inoperative in the steady state. Thus models of sieving based on interactions analogous to those observed in chromatography are unlikely to provide a basis for physical insight into the mechanism of steady-state sieving.

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Susceptibility of Mink to Sheep Scrapie

Abstract. *A progressive, fatal spongiform polioencephalopathy was induced in mink intracerebrally inoculated with a suspension of brain from a Suffolk sheep with naturally acquired scrapie. The clinical signs and pathological lesions of the experimental disease were indistinguishable from transmissible mink encephalopathy, a disease of undetermined origin that occurs in mink.*

Transmissible mink encephalopathy (TME) is an infection of ranch mink characterized by a long incubation period (minimum of 4 months on intracerebral inoculation) followed by a clinical course of progressive neurologic illness ending in severe debilitation and death. The principal histopathologic lesion seen in affected animals is a spongiform degeneration of the gray matter of the brain.

Epizootiologic evidence suggests that the rare natural occurrence of TME in mink herds results from the introduction of the disease agent through some item in the animal's diet. Clinically and pathologically, TME is very similar to scrapie of sheep. Studies on the physical and chemical properties of the TME agent have failed to differentiate it from the transmissible agent producing scrapie (*1*). However, since neither scrapie (*2*) nor TME (*3*) have been found to elicit a detectable immune response from their hosts, it has not been possible to relate the two diseases by means of immunologic procedures. Studies in experimental transmission between species have indicated that the etiologic agent of TME may in fact differ from scrapie in host range. Mink encephalopathy, in contrast to scrapie, has not as yet been demonstrated to be directly transmissible to Swiss white mice (*4, 5*) and, conversely, intraperitoneal inoculation of mink with mouse-adapted scrapie has failed to produce disease after 20 months (*5*). The TME agent has been transmitted to three species of subhuman primates (*6*) as well as to the raccoon and striped skunk (*7*). Various primates inoculated with mouse-adopted scrapie have failed to develop disease (*8*), while carnivore susceptibility to the scrapie agent is untested.

An experiment was designed to determine the susceptibility of mink exposed to the unadapted scrapie agent in tissues of infected sheep. Part of the continuing experiment is reported here. Brain homogenates were made from a purebred Cheviot and a purebred Suffolk sheep, respectively, each killed in an advanced stage of the naturally occurring disease. The diagnosis of scrapie was confirmed by pathologic examination and by mouse inoculation.

The inocula were prepared at Purdue University by trituration of frozen portions of brain tissue with the addition of enough physiological salt solution to produce a 10 percent suspension by weight. Five mink were inoculated (0.1 ml each) intracerebrally with the Suffolk homogenate and five with the Cheviot homogenate; the homogenates were also concurrently tested by intracerebral inoculation of mice. Mink used in the experiment were obtained in Wisconsin from ranch stock with no past history of TME and no known exposure to sheep tissues in their diet. At no time prior to transport to Purdue were the animals housed in facilities where research had ever been conducted on mink encephalopathy. The design of mink pens and holding shed, and the procedures for care and feeding, followed standards accepted by the mink industry. Inoculated animals were observed daily for signs of abnormal behavior.

The response of the mink inoculated with the Suffolk brain was uniform with the earliest signs of disease recorded 12 months after inoculation, and all five animals were affected after 14 months. The clinical signs consisted of behavioral changes, slowing of the animal's normal movements, postural ataxia, incoordination beginning in the hindquarters, and periods of somnolence with the mink apparently alert during the interim. The signs of disease were indistinguishable from those seen in natural (*9*) and experimental (*10*) mink encephalopathy. Mink inoculated with Cheviot brain remained normal 20 months after inoculation. Disease has never been produced in mink by the inoculation of normal mink brain nor have any uninoculated animals housed in the same sheds ever developed spontaneous disease. Both inocula induced scrapie in mice after incubation periods of 15 months for the Suffolk homogenate and 16 months for the Cheviot homogenate.

There were no gross pathological alterations in the central nervous sys-

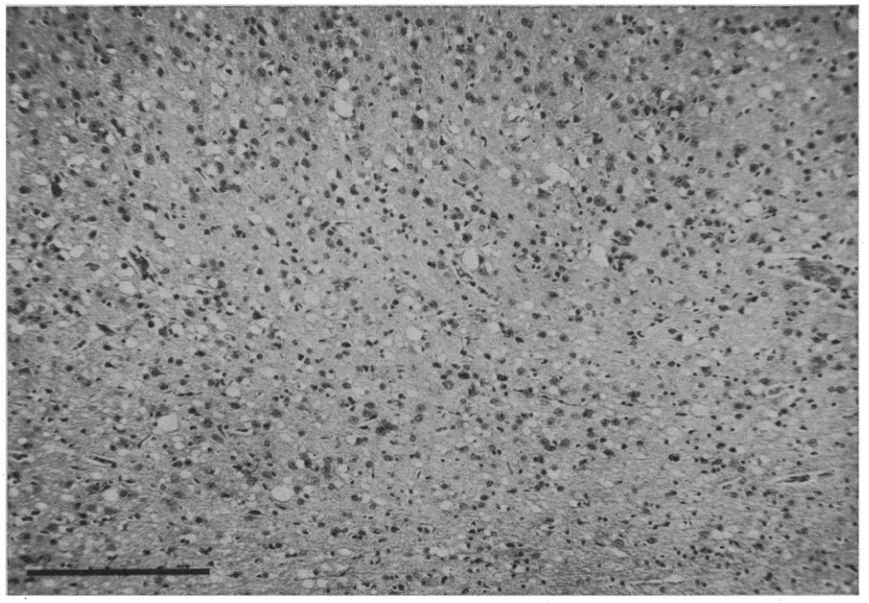

Fig. 1. Spongiform polioencephalopathy of cerebral cortex of mink inoculated intracerebrally with sheep scrapie. Hematoxylin and eosin stain; scale, 0.25 mm.

tem or the visceral organs of any of the diseased mink. In the examination, by light microscopy, of the brain from clinically affected mink, the most striking morphological alteration was a spongiform polioencephalopathy (Fig. 1). Vacuoles were located chiefly in the neuropil with an occasional vacuole in nerve cell bodies. The vacuoles were variable in size, the majority having diameters from 8 to 16 μm with occasional diameters up to 28 μm. Most were optically empty; however, some contained amorphous debris. Vacuolization was consistent and most intense in the cerebral cortex, corpus striatum, diencephalon, and mesencephalon and was consistent and less intense in the pons. In the medulla, vacuolization was not a constant finding and when present was less intense than in other parts of the brain. Extensive proliferation and hypertrophy of astrocytes was a constant feature in the areas of intense vacuolization. The lesions observed in the mink inoculated with scrapie are indistinguishable from those seen in the central nervous system of mink with transmissible mink encephalopathy (*5*, *9*).

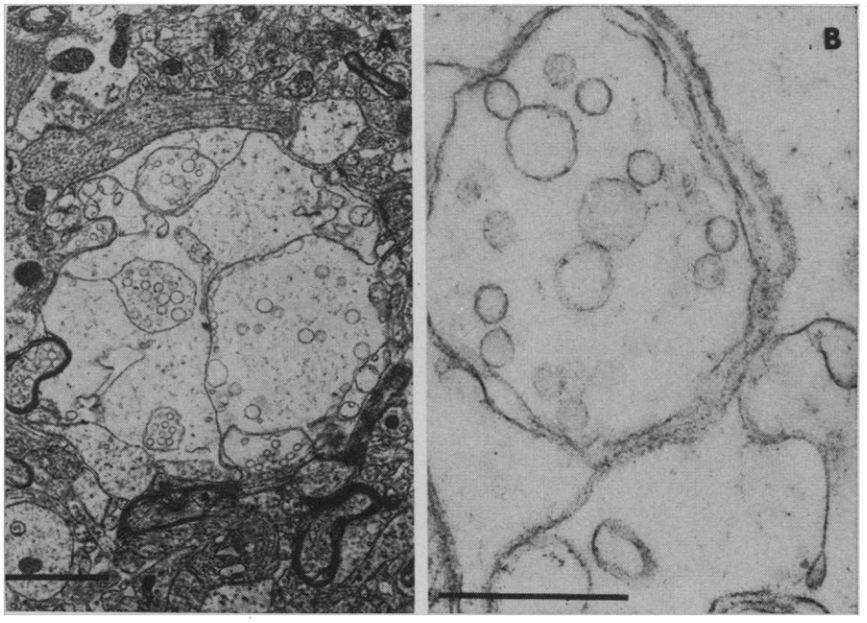

Fig. 2. Electron micrographs of cerebral cortex after immersion fixation of tissue in 3 percent glutaraldehyde, treatment in 1 percent osmium tetroxide, embedding in a mixture of Epon and Araldite, and contrasting of sections with uranyl acetate and lead citrate. (A) Round-shaped lesion of generally electron lucent appearance surrounded by processes of nerve cells and glial cells. Scale, 2 μm. (B) Detail of variously shaped membranes distributed in lesion. Scale, 0.5 μm.

Preliminary ultrastructural studies were performed on cerebral cortical tissues of two mink inoculated with scrapie-infected brain of the Suffolk sheep. Abnormal findings included the presence of large vacuoles in the neuropil which measured from 3 to 30 μm across, but most often were from 6 to 8 μm wide (Fig. 2A). These generally electron lucent areas were derived from distended and occasionally disrupted neighboring cell processes, some of them dendrites. Surface membranes, membrane fragments, and vesicular profiles from 100 to 200 nm across formed the chief electron opaque components (Fig. 2B). Also noted were focal and presumably hydropic swellings of axons and the presence of vacuoles up to 1.8 μm across within dendrites. These findings are consistent with those encountered in cortical tissues of TME-infected mink (*11*, *12*), hamsters (*12*), and squirrel monkeys (*6*).

There was a marked difference in the response of the mink to the Suffolk and Cheviot sheep inocula. The Suffolk brain produced disease in all inoculated mink over a 2-month period after an initial incubation of approximately 1 year. The Cheviot inoculum has produced no recognizable clinical disease, during a 20-month period of observation, in any of the five animals inoculated. There is accumulating evidence that strain differences do exist among scrapie agent isolates (*13*). It would appear that mink are susceptible to some "strains" of scrapie and that pathogenicity for mink is influenced, as it is for mice, by the breed of sheep from which it is derived.

These observations suggest that TME is a form of scrapie that results when mink become diseased after feeding on infected sheep tissues. This concept will be materially strengthened when sheep scrapie can be shown to be transmitted to mink by oral exposure. The host range of scrapie and TME may be determined by strain and

also in part by a factor that is derived from the host from which the inoculum is prepared. For this reason, we feel that it is important for investigators to reexamine primate susceptibility to the scrapie agent as it exists in sheep tissues.

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Temperature Tolerances of Some Closely Related Tropical Atlantic and Pacific Fish Species

Abstract. *Species of Pacific shallow-water fish are more tolerant of low temperatures than Atlantic species are. At high temperatures Atlantic species are more tolerant than Pacific species. For species pairs of* Bathygobius *differences in the tolerance of low temperatures are small and can be removed by acclimation to 23°C. Differences in the tolerance to low temperature in transisthmian species of* Apogon, *however, are large and persist after acclimation to 23°C. Some Pacific species adapt to the cooler temperatures of their habitat through increasing their rates of oxygen consumption at ambient temperatures or decreasing the dependence of oxygen uptake rate on temperature, or both.*

Atlantic and Pacific Panamanian shore fishes are useful for comparative studies of thermal tolerance because many species of the two oceans are closely related and the temperatures of the two oceans differ slightly. Phyletic affinities of the two faunas stem from late Pliocene times when, prior to the emergence of the Central American Isthmus, the Neotropical sea fauna was continuous (*1*). Physical oceanographic differences in the two oceans have been thoroughly studied; the eastern tropical Pacific has a lower average and more variable temperature as well as a more variable salinity than the tropical western Atlantic (*2*). Comparative studies of Atlantic and Pacific species make possible the determination of the effect of post-Pliocene differences in temperature on the temperature adaptations of particular species, and the relationship between thermal tolerance and taxonomic affinity in particular species pairs. Interest in comparative studies is further generated by the proposal for a Central American sea-level canal and by the need for assessing the colonizing capacities of different species (*3*).

As indices of temperature sensitivity, critical thermal (CT) maxima and minima were determined for three Atlantic and Pacific species pairs: the damselfishes (family Pomacentridae) *Abudefduf saxatilis* (Atlantic) and *Abudefduf troschelii* (Pacific), the cardinalfishes (Apogonidae) *Apogon maculatus* (Atlantic) and *Apogon dovii* (Pacific), and the gobies (Gobiidae) *Bathygobius soporator* (Atlantic) and *B. ramosus* (Pacific) (*4*). Critical thermal minima only were determined for a fourth species pair of soapfishes (Grammistidae) *Rypticus subbifrenatus* (Atlantic) and *R. nigripinnis* (Pacific). For determination of CT maxima and minima freshly collected groups of fish (of similar size) were heated or cooled from ambient seawater temperatures (26° to 28°C) in a filtered, aerated aquarium until they died. Rates of heating and cooling were regulated as precisely as possible and varied from 1.6° to 2.3°C per hour for heating and from 1.0° to 1.4°C per hour for cooling. In an additional experiment, species pairs of *Apogon* and *Bathygobius* were acclimated to 23°C for 15 days before determination of CT minima. Acclimation in the laboratory removes the effects of environmental variability and permits the determination of an organism's genetic capacity for thermal adaptation (*5*).

For all four species pairs, the Pacific species have significantly lower CT minima (Table 1) (*6*). The CT maxima of Atlantic fishes are significantly higher than those of the Pacific fishes (Table 1). The CT minima of the four species that had been acclimated to 23°C are significantly lower than those for unacclimated fishes (Table 2). Comparisons of Atlantic and Pacific fishes within this acclimated group show that *Apogon dovii* has a greater tolerance to cold than *Apogon maculatus* but that the CT minima of *B. ramosus* and *B. soporator* are not significantly different (Table 2).

The rate of oxygen uptake as a function of temperature was determined for three Atlantic and Pacific species pairs (*7*). Regression equations relating total oxygen consumption and wet body weight were formulated for each species at each temperature. These sets of equations were tested for interspecific differences in slope and position by F ratios. Regression line values of oxygen uptake from 15° to 35°C for 5-g individuals of *B. ramosus, B. soporator, Apogon dovii,* and *Apogon maculatus* are given (Table 3). Values for *R. nigripinnis* and *R. subbifrenatus* are line values for the regression of temperature on oxygen consumption for specimens in the size range of 0.9 to 3.4 g (Table 3). No measurements were made for *Abudefduf troschelii* and *Abudefduf saxatilis.*

From 20° to 35°C the rate of oxygen uptake of *Apogon dovii* is significantly