this degeneration was not a factor in our experiments. No evidence of such damage was observed in the eyes we studied, and the patterns of shedding and phagocytosis obtained with the OM rats were similar to those obtained by LaVail (*2*) in Fisher rats.

We have demonstrated that not only is the RCS pigment epithelium capable of phagocytosis, but that it normally phagocytizes small amounts of endogenous outer segment debris in a daily burst similar to that of the normal rat. The levels in vivo average about 5 percent of that of OM rats under comparable conditions. Furthermore, the phagocytosis can be increased in vitro to a level comparable to that in normal intact rat eyes before the burst of shedding (that is, about 5 phagosomes per 180 μm). The observations of residual bodies in the dystrophic pigment epithelium strongly suggest that not only does phagocytosis take place, but that the digestion of these phagosomes also occurs. Thus it is clear that the pigment epithelium of the RCS rat is impaired but not lacking in its ability to phagocytize the debris from the shed rod outer segments. This raises the possibility that the phagocytic rate could be manipulated (in vivo) to achieve normal levels and, thus, retard or prevent the progress of the retinal degeneration.

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8. Animals were anesthetized with sodium pentobarbital, the eyes were enucleated, and the anterior segments were removed in the dark for the dark-adapted animals and in the light otherwise. The eyecups were placed gently in Krebs-Ringer bicarbonate buffer with glycerol as the carbon source and incubated in the dark at 37°C in an atmosphere of 95 percent O_2 and 5 percent CO_2. After incubation for 1 hour the eyecups were transferred to RPMI-1640 medium (Gibco) to maintain the structural integrity of the retinas. The initial use of the Krebs buffer was dictated by an additional set of experiments on the same tissue, but not reported here.
9. Tissue was fixed either in 3 percent glutaraldehyde and 0.5 percent formaldehyde for 2 hours at room temperature or 1 percent glutaraldehyde and 1 percent formaldehyde overnight at 4°C. With both fixatives we used 85 m*M* phosphate buffer, *p*H 7.2. There was no change in phagosome counts that could be attributed to the effect of the fixative. Tissue was postfixed in 1 percent osmium tetroxide, dehydrated in a series of ethanol concentrations, and finally embedded in Araldite 502 resin. Thin (0.5 μm) sections were collected for light microscopy and sections of a silver interference color were collected for electron microscopy.
10. Phagosomes were counted across 290-μm lengths of pigment epithelium, the field of view of the 63X Zeiss Planapochromat oil immersion objective used for the counting. Phagosomes in OM rats were defined as densely staining bodies measuring at least one outer segment diameter in the smallest dimension and located either in the pigment epithelium somas or microvilli. Phagosomes in the RCS rats were defined as being completely contained within the pigment epithelium and having a minimum diameter of at least 1 μm. Although the diameter of an outer segment was found to be 1.5 μm, these bodies are inserted into the pigment epithelium at an angle, so that the projection of an average outer segment onto the pigment epithelium was 1.8 ± 0.3 μm (based on measurements of 37 eyes). Counts are reported as phagosomes per 180 μm, which approximates phagosomes per 100 outer segments.
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Virulent *Naegleria fowleri* in an Indoor Swimming Pool

Abstract. *A reservoir of pathogeniw* Naegleria fowleri *has been located in the cracked wall of a swimming pool where repeated outbreaks of primary amoebic meningoencephalitis were observed between 1962 and 1965.*

An indoor swimming pool in northern Bohemia proved to be the source of the infectious agent in four repeated outbreaks of primary amoebic meningoencephalitis (PAME) between 1962 and 1965 (*1*). In epidemiologic investigations the actual location where pathogenic *Naegleria* survived and multiplied to critical concentrations was not traced. The system of water recirculation and technology was improved, and neither *Naegleria fowleri* nor further cases of PAME occurred in the following years (*2*).

After a 12-year interval, in the winter of 1977 to 1978, several strains of *N. fowleri* highly virulent to laboratory mice were isolated from samples of water from this swimming pool. For new examinations the method by Červa (*3*) as modified by Griffin (*4*) was used (*5*). During 3 months 23 strains of *N. fowleri* were found. The first five strains were isolated from the water near the steps, the bottom of the swimming pool, and the surface of the filter in the recirculation system.

Renewed investigations revealed that the inside front wall in the deep part of the pool was cracked in several places. It was found that this wall had been built many years ago to adjust the length of the swimming pool to exactly 25 m. The isolating layer between the main concrete wall of the swimming pool and the inside tiled surface (Moniér's wall) cracked and a layer of free air approximately 10 cm thick resulted. Later the cracks in the facing enabled water to flow between these walls. The temperature in this space was between 27° and 30°C and the water there was relatively isolated from the influence of any disinfectants. Rich populations of pathogenic *Naegleria* developed on the inner walls of the confined area.

For the purpose of competitive swimming, at weekly or monthly intervals, the water level was raised to about 40 cm above the usual level maintained for recreational swimming. We assume that this periodic fluctuation of the water level flushed some of the pathogenic amoebas from the space between the two walls and that these amoebas then infected the swimmers.

Thick layers of organic material containing *N. fowleri* were found on the walls of the confined space. Eighteen strains of *N. fowleri* pathogenic for mice were isolated from samples of this material.

In our opinion this source of the infectious agent survived from the time of the first epidemic occurrence of PAME in 1962. This space served as a reservoir for the *Naegleria* until it was found years later when the cracks in the wall surface became more obvious. Reconstruction of the wall should remove the risk of occurrence of PAME among swimmers in this pool in the future.

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