periment was carried out with RDP, which is pronase-resistant.

At stage 6a, prior to the onset of n bandlet stem cell production, an N teloblast was injected with RDP. At stage 7, after stem cell production was under way, the same N teloblast was reinjected with pronase; Fig. 2f shows such an embryo at 9 days of age. The anterior part of the nerve cord of this embryo consists of morphologically normal (bilaterally symmetric) ganglia, whereas the posterior part consists of abnormal (bilaterally asymmetric) ganglia deficient in cell number on the side of the ablated N teloblast. Moreover, anterior ganglia contain neurons labeled with RDP, whereas posterior ganglia do not. The boundary between anterior morphologically normal and posterior morphologically deficient ganglia coincides with the boundary between anterior RDP-labeled and posterior unlabeled ganglia. Thus it follows that the anterior ganglia of the pronase-treated embryo contain progeny of n stem cells produced prior to ablation of the N teloblast and that the posterior ganglia are abnormal because they received no cellular contribution from the N teloblast.

The fluorescent peptides RDP and FDP have further advantages as cell lineage tracers: (i) The red-fluorescing RDP and the yellow-fluorescing FDP can be used in combination for double-label experiments; and (ii) their distribution can be observed in living embryos, in contrast to that of HRP, which can be visualized only in fixed preparations. Thus these fluorescent tracers should make it possible to follow the appearance of successive descendants of an injected early embryonic cell in the same preparation and to know the embryonic origin of nerve and muscle cells identified by intracellular electrophysiological recordings.

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31 December 1979; revised 22 February 1980

Naegleria fowleri: Trimethoprim Sensitivity

Abstract. *Trimethoprim in a concentration of 4 micrograms per milliliter of Bacto-Casitone (Difco) medium inhibits the growth of nonvirulent* Naegleria fowleri *isolates. The growth of virulent strains is unaffected even with 400 micrograms of the drug per milliliter of medium. Differences in sensitivity constitute the possibility of a simple selection of environmental isolates. The pathogenicity and virulence of* Naegleria *species may be connected with the metabolism of folic acid.*

In an attempt to find simple markers by which we could differentiate among the various species, strains, or variants of amoebas of the genus *Naegleria* we tested—among other factors—the effect of several chemotherapeutics on the growth of *Naegleria fowleri* in axenic cultures. We observed that some strains were inhibited under defined conditions by low concentrations of trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine].

We used a total of 31 strains of *N. fowleri* in the experiments. Ten of them were isolated from human cases of primary amoebic meningoencephalitis and 21 strains were isolated from water samples of warm industrial effluents in Czechoslovakia. Three of these environmental strains were pathogenic for laboratory animals. The basic tests were carried out in tubes containing 5 ml of fluid BCS medium, that is, 2 percent Bacto-Casitone (Difco) in distilled water with 10 percent of fresh rabbit serum (*1*). Trimethoprim (*2*) was added to this medium in concentrations ranging from 0.4 μg/ml to 400 μg/ml. The tubes inoculated with the different strains of amoebas were incubated at 37°C.

All the nonvirulent isolates of *N. fowleri* were completely inhibited by trimethoprim in concentrations of 4 μg/ml and higher. The strains isolated from humans and the virulent strains from the industrial effluents tolerated the highest tested concentrations of the drug without any definite changes of growth rate. Several other antagonists of folic acid such as aminopterin, 3,5-diaminopterin, and methotrexate (*3*) applied in concentrations up to 50 μg/ml under identical experimental conditions did not affect the growth of any of the *N. fowleri* strains.

The inhibitory effect of trimethoprim on nonvirulent environmental strains could be prevented by addition of folic acid or leucovorin (*3*) to the medium in concentrations of 50 μg/ml. However, 2-amino-4-hydroxy-6-(tetrahydroxybutyl)-pteridin (*3*) had no antagonistic effect on trimethoprim. Culture media containing such ingredients as liver digests or extracts, yeast extracts, peptones, or a suspension of thermally killed bacteria are inconvenient for experiments with trimethoprim activity because of their folic acid content.

The effect of trimethoprim on *N. fowleri* in BCS medium provides a simple and reliable method for differentiation of virulent and nonvirulent strains of this organism isolated in ecological and epidemiological environmental studies. Further examination of the differences in the metabolism of folic acid in *N. fowleri* may help to elucidate the conditions of pathogenicity and virulence of *Naegleria* species.

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28 March 1980

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