slight headaches in the evening of 25 June, increased temperatures (38.5°C) in the evening of 26 June, and meningeal syndrome on 27 June, whereupon he was hospitalized. Peracute development of purulent meningitis ended with central nervous system failure in the evening of 29 June. Intensive therapy (penicillin, chloramphenicol, erythromycin, and Gantrisin) had no favorable effect on the course of the disease. No indication of amoebic etiology was expressed during the course of the disease. Postmortem examination revealed amoebae in the brain, although the nasal swab culture of this patient remained negative.

The patient had bathed in a brook and in an open swimming pool in Most (Northern Bohemia) shortly before the onset of the disease. These localities may be sources of the infective agent. No connection was found with the previously described focus of a similar pathogenic amoeba in the town Ústí nad Labem (*1*). No similar disease appeared among several hundred other persons bathing in the same localities within the same period as the deceased. In water samples of both the brook and swimming pool a relatively rich population ($10^3$ to $10^5$ cell/liter) of limax amoebae was found. No strains pathogenic to mice were isolated from the water samples, however.

The amoeba strain isolated from the spinal fluid of the deceased boy probably belongs to the genus *Naegleria*. It produces swimming stages with two flagellae in a liquid milieu and produces large numbers of characteristic disk-like cysts on the agar slants. Amoebae of this strain are a little smaller than the HB-1 strain of *Naegleria* isolated from man in the United States (*3*) and differ in other morphological details, too. Pathogenicity for mice and guinea pigs after intranasal application of this strain was proved.

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**References**

1. L. Červa and K. Novák, *Science* **160**, 92 (1968).
2. C. G. Culbertson, P. W. Ensminger, W. M. Overton, *Amer. J. Clin. Pathol.* **43**, 383 (1965).
3. C. G. Butt, C. Baro, R. W. Knorr, *ibid.* **50**, 568 (1968); C. G. Culbertson, P. W. Ensminger, W. M. Overton, *J. Protozool.* **15**, 353 (1968).

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## Amoebic Meningoencephalitis: Axenic Culture of Naegleria

Abstract. *A medium consisting of 2 percent Bacto-Casitone (Difco) and 10 percent fresh horse serum in distilled water ensures axenic growth of two pathogenic isolates of* Naegleria *species.*

A simple modification of the Bacto-Casitone medium (*1*) designed originally for *Acanthamoeba* (*Hartmannella*) *castellanii* can be used for axenic cultures of the pathogenic strains of *Naegleria* sp. Composition of the medium is Bacto-Casitone (Difco), 20 g; distilled water, 1000 ml; and sterile fresh horse serum, 100 ml. If necessary 500 I.U. of penicillin and 50 μg of streptomycin per milliliter can be added.

In this medium both the American HB-1 (*2*) and Czech (*3*) strains of pathogenic *Naegleria* sp. give a rich growth, mainly on the walls of the test tubes. Flagellated stages are formed in large numbers especially by the Czech strain, even in older cultures. Optimum transfer interval is approximately 5 days; occasionally successful transfers can be accomplished after 10 or 15 days.

Several strains of *Acanthamoeba* in our collection which do not grow satisfactorily in media without bacteria are easily cultured in this medium, too. No differences in the growth of amoebic cultures were observed in media with rabbit, calf, and horse serums. The growth factor is thermolabile. Inactivated serums are inconvenient for the preparation of this medium.

This axenic culture method provides mass production of a relatively pure amoebic antigen for immunological purposes and simplifies some other laboratory and diagnostic procedures.

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**References**

1. L. Červa, *Folia Parasitol. (Praha)* **13**, 328 (1966).
2. C. G. Culbertson, P. W. Ensminger, W. M. Overton, *J. Protozool.* **15**, 352 (1968).
3. L. Červa, V. Zimák, K. Novák, *Science*, this issue.

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## Hyperbaric Oxygen: Toxicity to Fish at Pressures Present in Their Swimbladders

Abstract. *When juvenile Pacific rockfish,* Sebastodes miniatus, *are exposed to oxygen tensions equal to those in their swimbladders, they exhibit symptoms characteristic of oxygen poisoning in mammals and ultimately die. Thus their central nervous system appears to be as sensitive to elevated oxygen pressure as that of higher vertebrates, whereas the cells of the gas gland tissue inside the swimbladder must be insensitive to the partial pressure of oxygen which they help to produce.*

Although a number of enzymes taking part in the citric acid cycle and glycolysis are inhibited by high oxygen pressure (*1–3*), the gas gland tissue in the swimbladder of marine fish functions under oxygen pressures exceeding 200 atm (*4–6*). This must represent a localized adaptation, since oxygen is toxic to the central nervous system of vertebrates (*3, 7–9*). However, the degree and nature of the adaptation is not known.

In general, an organism's sensitivity to high oxygen pressures bears a direct correlation to its dependence on oxygen (*10, 11*); one that can tolerate anaerobiosis is more resistant than others which cannot. This is consistent with the fact that high oxygen pressure inhibits aerobic energy metabolism (*1–3*).

Inasmuch as some fish display marked resistance to anoxia (*11*), it is of interest to determine their susceptibility to high oxygen pressure. During investigation of glycolysis of gas gland tissue from *Sebastodes miniatus*, one of the common rockfish of the Pacific coast, I measured the $O_2$ sensitivity of the intact fish over a range of oxygen pressures known to exist in their swimbladders (*12*).

Juvenile *S. miniatus* were captured with scuba at depths from 10 to 40 m. Quinaldine was used as an anesthetic. Fish were kept in shallow running seawater for up to 2 weeks before an experiment and were fed liberally with frozen brine shrimp during that time.

Individuals (2 to 7 g) were placed in seawater contained in a Lucite-lined stainless steel pressure chamber fitted with a Lucite top 2.5 cm thick (Fig. 1); this chamber was equipped with three ports which accepted a gas inlet, a gas outlet, and a static line to a pressure gauge. The gas (oxygen or compressed