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.

L. Červa
V. Zimák
K. Novák

Army Institute of Hygiene, Epidemiology and Microbiology, Prague, Czechoslovakia

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24 September 1968; revised 12 November 1968

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.

L. Červa

Army Institute of Hygiene, Epidemiology and Microbiology, Prague, Czechoslovakia

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12 November 1968

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

air) was supplied from a high-pressure cylinder through a two-stage regulator, and the gas outlet was equipped with a micrometer needle valve. By adjustment of both the second stage of the regulator and the needle valve, a minimum flow rate (sufficient to produce a very low partial pressure of CO_2 at any desired partial pressure of O_2) was maintained. Seawater constituted approximately 60 percent of the volume of the chamber; the remainder was gas. Analysis of this gas at 2 atm absolute showed less than 0.10 percent CO_2 when the flow rate of gas was 10 cm^3/min. In order to provide efficient equilibration and CO_2 exchange, the entering gas was dispersed through a sintered glass candle.

All experiments were carried out at 15°C. Once in the chamber, fish were observed at hourly intervals whenever possible. Fish were kept in the chamber until dead, unless they survived for more than 5 days, in which case the experiment was terminated so as to minimize the possible effects of starvation (*13*).

Symptoms resulting from high oxygen pressure and the sequence of events preceding death are similar to those observed in mammals, except that the degree and rate of onset are somewhat less predictable at any given oxygen pressure. The initial sign of distress was a rigid display of all fins and dorsal spines; this distress became more extreme whenever the chamber was lightly tapped or when the fish was disturbed in some way, for example, by a small light shining upon it. This rigidity of fins and dorsal spines gradually became permanent, as if the control of the muscles involved was gradually lost. In this condition the fish either rested on the bottom of the chamber or floated, usually inverted, at the surface. The ventilation rate gradually increased, and often the color pattern of the fish was accentuated, with exceptionally high contrast. As the fish became more disturbed, paralysis of body muscles became evident, and the swimming motions were more laterally restricted, less supple, and spasmodic. Swimming gradually became violent and uncontrolled; the fish began to lose orientation control and swam or rested in an inverted position on the bottom of the chamber. When fish were under a pressure of approximately 2 atm absolute or less, they usually became buoyant during the onset of these symptoms, indicating some secretion of gas (*14*). As the toxic effects progressed, the depth and rate of ventilation increased for a period of time (which was variable in different fish) and then began to decrease. Usually, as breathing slowed and became weaker, the pectoral fins became rigid. After this loss of function the gills continued to work, but with greater restriction. Immediately before cessation of inspiration, both gills and eye muscles were often subject to spasmodic tetanic contractions. Death, as indicated by cessation of ventilation, followed shortly. These observations demonstrate that symptoms of O_2 poisoning are similar in fish and mammals, but the onset of neurological symptoms characteristic of the acute phase of O_2 toxicity is more gradual in fish. Furthermore, the convulsive seizures usually precede muscular paralysis in mammals, whereas in this species of fish seizures tend to occur intermittently as paralysis sets in.

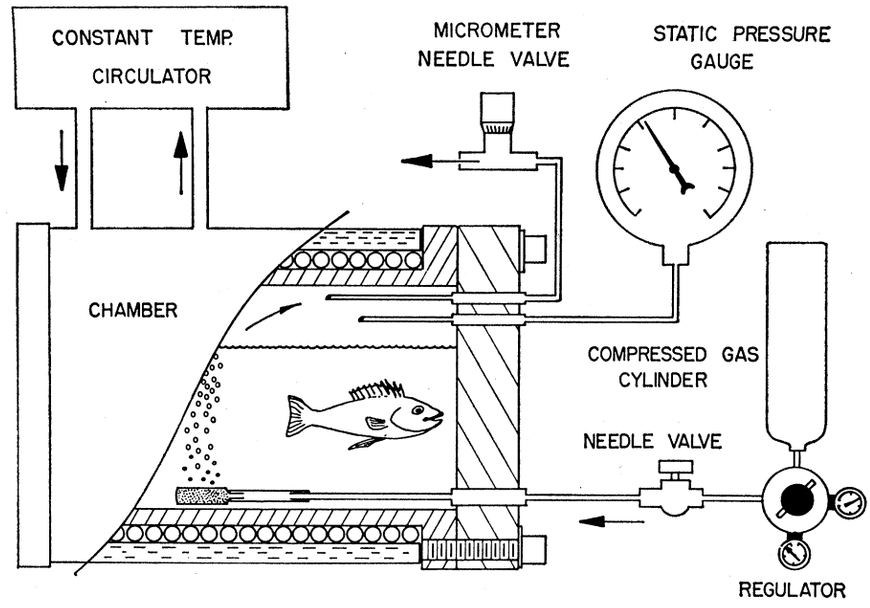

Fig. 1. Experimental apparatus.

Figure 2 summarizes the results of the exposure of fish to a range of oxygen pressures. The curve is similar to those of Marshall and Lambertsen (*7*) and Gerschman (*8*), which show for mice time to onset of convulsions and time of survival, respectively, as a function of oxygen pressure. The survival time for this species of fish (Fig. 2) is greater than that in mice over the entire range of oxygen pressures used. The lower temperatures (15°C) are partly responsible for this difference, although the extent is not evident from this data; nor is it advisable to estimate the effect of temperature on the basis of an arbitrarily assigned temperature coefficient. However, the possibility of investigating the effects of temperature on the "rate constant" of death from hyperoxia by use of poikilotherms, such as fish which could be acclimated over a range of temperatures, may be useful as a means of separating different classes of toxic effects. These results show that this species of fish cannot tolerate the oxygen pressures which they are able to produce and maintain in their swimbladders. To the extent that the metabolic pathways or subcellular systems

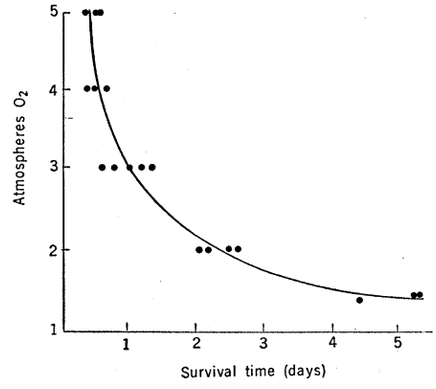

Fig. 2. Survival time as a function of oxygen pressure in juvenile *Sebastodes miniatus* exposed to a range of oxygen pressures. In order to rule out the effects of hydrostatic pressure, the lowest partial pressure of oxygen (1.5 atm absolute) was also administered to fish in the chamber as compressed air, so that the total pressure was 7.5 atm absolute. There was no significant difference in the survival time of fish treated with these two gas mixtures.

functioning in gas gland tissue are present in the central nervous system, such pathways are less likely to be directly involved in the acute phase of oxygen toxicity.

Haugaard (*1*) has emphasized some chemical effects of high oxygen pressure that require further study; among these are lipid peroxidation and oxidation of —SH groups. It is unlikely that gas gland tissue is sufficiently unique to be independent of these very general effects of oxygen.

The fact that the energy metabolism of this tissue is primarily glycolytic is in accord with the fact that aerobic pathways are sensitive to high oxygen pressure (*1, 2*). Although enzymes taking part in the citric acid cycle (several of which are known to be reversibly inactivated by high oxygen pressure with a time constant approximating that of convulsions in the intact animal) (*15*) have been demonstrated in the gas gland tissue of the eel (*Anguilla*) and the cod (*Gadus*) (*5*), they are apparently weakly represented in the gas gland tissue of *S. miniatus*. Very low recoveries of C^{14}-labeled CO_2 were obtained when this tissue was incubated with uniformly labeled glucose under oxygen at pressures from 0 to 50 atm absolute (*12*).

Thus, fish possessing cells which are essentially insensitive to oxygen are as sensitive as mammals to high oxygen pressure. The insensitivity to oxygen of the glycolytic system of the gas gland tissue remains unexplained since either of the above mentioned mechanisms could potentially inhibit or arrest glycolysis at sufficient O_2 pressure.

BRIAN G. D'AOUST*
Physiological Research Laboratory, University of California, San Diego, P.O. Box 109, La Jolla 92037

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* Present address: Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014.

12 December 1968

Collagen Has a Discrete Family of Reactive Hydroxylysyl and Lysyl Side-Chain Amino Groups

Abstract. *Several cross-linking substances which derive from lysine have been isolated from hydrolyzates of collagen and elastin. This observation suggests that certain lysyl side chains exhibiting enhanced reactivity and unique configuration may participate in cross-linking. In native collagen there is a small family of lysyl and hydroxylysyl side chains which exhibit a high propensity of Schiff base formation.*

As connective tissues mature, acquiring a high degree of tensile strength, covalent interchain cross-links are introduced within the fibrous proteins. Because aging and several important human disease states may be related to defects in this process of maturation (*1*), the nature of the cross-linking substances has been intensively studied. Compounds which serve as covalent interchain cross-links in elastin and the intramolecular cross-link of collagen (*2*) arise by condensation of the side chains of strategic lysyl residues (*3*) after enzymatic oxidative deamination (*4*). However, the nature of the intermolecular cross-link of collagen has not yet been determined. It has been postulated that this cross-link too belongs to the lysyl-derived family, and there is evidence that the cross-link may be a Schiff base formed by condensation of a reactive lysyl side chain with an aldehyde function of an adjacent molecule (*5*). Thus, soluble collagen might be expected to have a small family of exceptionally reactive lysyl side chains which are available for Schiff base formation with an appropriate reagent.

Pyridoxal phosphate may be the reagent of choice for marking these lysyl side chains. This compound reacts with both enzymatic and nonenzymatic proteins in a specific way to form a Schiff base with certain lysyl side chains (*6*). The evidence indicates that pyridoxal phosphate may be present at the active site of the enzyme which normally oxidizes these cross-linking lysyl residues (*7*) and therefore might be expected to have a high affinity for them. As a result of the characteristic absorbance and fluorescence spectra, very small amounts of the compound can be detected in proteins. Thus, it appeared that the reaction between pyridoxal phosphate and collagen might provide a way in which those lysyl side chains destined for participation in collagen aggregation and maturation might be selectively marked, thereby permitting determination of their location and nature. Indeed, we found that a small family of reactive lysyl and hydroxylysyl groups is available for reaction with the aldehyde function of pyridoxal phosphate.

Collagen was extracted in 1*M* NaCl from the skin of growing rats and purified and lyophylized (*8*). Collagen solutions (4 mg/ml) for reaction with pyridoxal phosphate were prepared in 0.5*M* NaCl containing 0.05*M* phosphate at *p*H 7.4. Pyridoxal phosphate (*9*) was prepared in the same buffer at a concentration of 16 μmole/ml. A solution of sodium borohydride (10 mg/ml) was made up in water. The buffers were perfused with nitrogen, and reagents were always made up at the time of use. The reaction was carried out at 4°C on mixtures containing 2 ml of collagen solution, 0.16 to 32 μmole of pyridoxal phosphate, and buffer to make a final volume of 4 ml.

After completion of the reaction, the mixture was reduced by the addition of 0.15 ml of sodium borohydride solution; stirring was continued, and additional portions of sodium borohydride (0.15 ml) were added after 15 and 30 minutes. The mixtures were stirred for an additional hour and dialyzed against 0.1*N* acetic acid until no further re-