Table 1. Time for germfree and ex-germfree vitamin A-deficient rats to reach a weight plateau and their periods of survival.

Type and sex	No. of rats	Weight plateau		Survival time (days)
		Time (days)	Weight* (g)	
		First experiment		
Germfree ♂	4	45–74	192–265	145–202†
Germfree ♀	4	67–135	187–225	170–272†
Ex-germfree ♂	3	32–35	144–186	42–46
Ex-germfree ♀	2	18–25	122–132	23–45
		Second experiment		
Germfree ♂	5	28–32	163–217	56–150‡
Germfree ♀	5	28–41	160–189	58–150‡
Ex-germfree ♂	5	23–28	154–195	32–54
Ex-germfree ♀	5	28–35	141–204	34–50

* Average weaning weights of males and females were, respectively: first experiment, 30 and 25 g; second experiment, 47 and 45 g. † Not all rats died; some were cured by feeding retinyl acetate. ‡ After 150 days, four males and one female were still alive.

pronounced for about 1 week, then underwent no further change. At this time, there was depilitation around the eye (so-called "spectacled eye") but no porphyrin pigment was apparent. Germfree, vitamin A-deficient female rats showed similar growth retardation and nervous symptoms 2 to 8 weeks later than the males.

The deficient, germfree rats, after reaching a weight plateau, generally maintained a constant weight for prolonged periods. The only change in condition was an accumulation of porphyrin around the eyes. Eventual death was always preceded by weight loss for about 2 weeks and cessation of eating. An autopsy showed that all rats had urinary bladder stones. Death was attributed to intestinal strangulation (volvulus) in three rats, urinary blockage in two, and was undetermined in two. None of the germfree rats supplemented with vitamin A died, but grew and appeared normal in all respects.

In the second experiment [young rats whose mothers were fed the vitamin A-deficient diet during lactation], the deficient rats and the vitamin A-supplemented control rats were kept in separate isolators to preclude the possibility of the deficient rats having access to traces of vitamin A. These rats were considerably heavier at weaning than in the first experiment, presumably because their mothers were changed to the deficient diet during the lactation period rather than before the young were born. Both male and female germfree deficient rats reached a plateau earlier than in the first experiment (Table 1), probably because of a faster growth rate, and they died sooner. Causes of death, when ascertained, were the same as in the first experiment. After 150 days, five rats were still alive and were maintaining weight.

To test the possibility that the casein in the deficient diet may contain a trace of vitamin A that could be sufficient for survival in the germfree state, the 22 percent casein was replaced with a mixture of 4 percent casein and 10 amino acids, to give an essential amino acid composition equivalent to that of 18 percent casein (*3*). Two germfree rats, deficient for 126 days, were fed this diet for 3 weeks. After an initial adjustment period of 1 week, during which slight weight loss occurred, they maintained weight for 2 weeks with no change in appearance.

One germfree deficient rat whose weight (256 g) had been constant for 5 weeks was given retinoic acid in the diet (12 mg/kg). Weight gain began in 3 days and continued for 14 days when the retinoic acid was withdrawn (weight, 304 g). No change in weight occurred during the next 30 days. A second addition of retinoic acid to the diet for 14 days promoted a weight gain of 24 g. When the supplement was withdrawn, the weight gain again ceased promptly, and a constant weight was held for the next month.

These experiments indicate that vitamin A is not essential for prolonged survival (*4*) of the germfree rat that has been weaned with low tissue stores of the vitamin (*5*). Early death in conventional deficient rats must be a consequence of bacterial infection.

Note added in proof: In a third experiment, the vitamin A-deficient diet had an L-amino acid mixture substituted for casein and sucrose substituted for starch. The diet was sterilized by irradiation. Three ex-germfree deficient rats died after 46 to 54 days. One germfree deficient rat died on the 68th day and three others were still alive after the 100th day.

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1. D. L. Beaver, *Amer. J. Pathol.* **38**, 335 (1961).
2. Composition of the diet (percent): vitamin-free casein (Nutritional Biochemicals Corp.), 22; Wesson salt mix, 4; corn oil, 4; vitamin mix in sucrose, 2; cornstarch, 68. The vitamin mix had three times the normal amounts of all vitamins except A.
3. This diet could be autoclaved satisfactorily, whereas a diet containing 19 L-amino acids and no casein became dark brown and hardened on cooling.
4. Drs. N. Raica and H. E. Sauberlich of the U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado, have also observed prolonged survival in germfree, vitamin A-deficient rats (personal communication).
5. Analysis of livers from two rats when weaned for the second experiment gave 5.6 and 8.2 μg of vitamin A per liver.

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Amoebic Meningoencephalitis: A New Amoeba Isolate

Abstract. *A strain of* Naegleria *sp. was isolated repeatedly from the spinal fluid of a boy who died of acute meningoencephalitis 5 days after the onset of the first symptoms.*

Since 1962 sixteen cases of fatal amoebic (limax-type) meningoencephalitis have appeared in Northern Bohemia (*1*). The causal agents were detected in histologic preparations, but never isolated in culture.

In 1968 spinal fluids and nasal swabs of all patients with the meningeal syndrome hospitalized in the department of neuroinfections of the North Bohemian district hospital were cultivated on agar slants [2 percent weight by volume of Bacto-Agar (Difco) in distilled water] coated with a suspension of thermally killed *Aerobacter aerogenes* culture before the beginning of therapy. The ability of our strain of *Aerobacter aerogenes* to support the growth of *Naegleria* even after the thermal preparation described (*2*) was also demonstrated with the pathogenic HB-1 strain of *Naegleria* sp.

Positive cultures were obtained from three spinal fluid samples collected on 27, 28, and 29 June 1968 from a 12-year-old boy. This patient developed

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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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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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