length of the leaf rhythm under different light intensities may just be a unique response of Pinto beans. However, since it does exhibit a persistent rhythm under continuous high-intensity light, the Pinto bean may be useful in further studies on the nature of circadian rhythms in plants.

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Hyperbaric Exposure of Mice to Pressures of 60 to 90 Atmospheres

Abstract. *Albino mice breathing helium-oxygen mixtures remained in good condition for periods of 1 to 13 hours at pressure-equivalent depths of 396 to 914 meters of sea water. They were successfully decompressed to normal atmospheric pressure in less than 5 hours. There were no immediate or delayed adverse effects that could be attributed to the hyperbaric helium-oxygen environment. An occasional death was attributable to hypoxia or the decompression procedure.*

Our objective was to obtain a respirable synthetic atmosphere for mammals which would be adequate for maintaining normal physiological functions at very high ambient pressures.

Marshall (*1*) reported that mice began to show a loss of equilibrium at nitrogen pressures of 10 to 17 atm. Helium, however, at 41 atm caused no signs of equilibrium loss in mice exposed for periods up to 2 hours. When the pressure was raised to 54 atm (helium), mice became stuporous in about 40 minutes, but recovered completely after decompression. From data of Carpenter (*2*) the "isonarcotic" concentrations of nitrogen and helium required to protect mice against electroseizures are 18 and 163 atm, respectively (*3*). Translated into impairment produced by physiologically inert gases at depths tolerated by divers, it appears that helium might have approximately a ninefold advantage over nitrogen. At 10 atm (91.4 m) divers breathing air usually show varying degrees of impairment, and at 15 atm (137.2 m) they show loss of useful function within a matter of minutes. In a helium-oxygen atmosphere, comparable degrees of impairment might not appear until the pressure reaches 60 to 90 atm. Limiting diving depths in pressurized atmospheres are generally estimated to be at a much lower range of about 305 to 427 m. In view of the renewed interest in exploration of the Continental Shelf and problems related to deep submarine submergence, it seemed appropriate to initiate preliminary tests with mice to observe reactions at depths equivalent to 610 to 914 m (60 to 90 atm). A proper decompression procedure is especially important in such experiments.

Helium was chosen as a substitute for nitrogen because of the well-known narcotic properties of nitrogen (*4*) and because of the proven effectiveness of helium-oxygen mixtures at pressures unattainable by a diver breathing air (*3*).

There is evidence that increased oxygen tension rather than barometric pressure itself is responsible for the morbidity and mortality which occurs under conditions of prolonged exposure to air at high-pressures (*5*). Workman *et al.* have shown that rats and monkeys exposed to synthetic atmospheres of helium and oxygen under conditions of pressure equivalent to 61 m of sea water for periods of two weeks tolerated the procedure without deterioration (*6*). The oxygen tension in their experiments was controlled at 3 percent of the total helium-oxygen pressure at 7 atm absolute. This is equal to 0.21 atm and equal to that found in air at 1 atm.

Although oxygen at 0.21 atm is probably adequate even under pressure equivalent to a depth of 1000 m, we attempted to keep the oxygen tension throughout the wide range of pressures within 0.21 and 0.60 atm. The upper limit of oxygen tension used (0.6 atm) is considered to be the upper limit of oxygen tension which is safe for human exposure for prolonged periods of time (*7*).

Mice were considered especially suitable for these experiments because of their small size and consequent rapid uptake of inert gas during compression, and subsequent rapid elimination of the gas during decompression. It has been estimated that mice attain gaseous equilibrium within 1 hour of being placed in the hyperbaric environment, in contrast to man who requires 12 hours to attain equilibrium.

The test compartment was a small pressure chamber hydrostatically tested to 191 atm. The chamber measured 30.48 cm in length and 15.24 cm in diameter and had a volume of 805.18 cm^3. The chamber was provided with internal illumination, and portholes in each side enabled us to observe the mice. A thermocouple was used for measuring temperature. The oxygen content of the chamber was measured when the oxygen tension was high enough to be within range of the instrument used (*8*) (above 1 percent). Baralime was placed in the chamber to aid in the absorption of carbon dioxide. During most of the experiments in which the pressure was equivalent to a depth of 610 m of sea water, a small exercise wheel was placed in the chamber and used actively by several mice.

In our first experiment, two mice were placed in the pressure chamber which was purged with oxygen. The pressure was raised to 1.16 atm (absolute) with a mixture of 25 percent oxygen and 75 percent helium to provide an oxygen tension slightly higher than that found in air at 1 atm. Subsequently, the pressure was raised to 42 atm absolute, or pressure at a depth of 412 m of sea water over a period of 30 minutes, pure helium being used as the additive. The oxygen tension was maintained at approximately 0.3 atm. The mice were kept at this pressure for 13 hours and 40 minutes, then gradually decompressed to normal atmospheric pressure. They tolerated the procedure well and survived with no immediately apparent physiological deterioration. However, 1 week after the experiment one mouse died. Gross examination revealed several milliliters of serosanguinous fluid in the abdominal cavity. Histopathological studies (*9*) showed normal lung tissue. The liver showed definite lymphatic dilatation, areas of separation of tissue, and dilatation of the central veins. Another mouse in the same cage, which had not been subjected to increased pressure, died at the same time, suggesting an infectious process as the cause of death.

In this and subsequent experiments it was observed that the mice began shivering when the temperature inside the chamber dropped below 31.8°C. When one side of the chamber was heated, the mice migrated to that side and appeared comfortable.

For the next test, two mice were placed in the chamber, which was then purged with oxygen, and the pressure was increased to 1.68 atm with a mixture of 25 percent oxygen and 75 percent helium. The pressure was raised with pure helium to 62 atm absolute, equivalent to a depth of 615 m of sea water over a period of 45 minutes. After 1½ hours, a sample of gas was taken from the chamber and analyzed for oxygen and carbon dioxide (*10*). The percentage of oxygen was 0.62 and of carbon dioxide was 0.08, indicating an oxygen and carbon dioxide tension of 0.38 and 0.05 atm, respectively. The mice were active and appeared alert during the 7-hour period they were observed. Due to an error the mice were kept an additional 8 hours at the pressure of 62 atm without adding oxygen or ventilation. They were dead at the end of that time, and the cause of death was presumed to be anoxia or carbon dioxide poisoning. Histopathological studies (*9*) of the mice revealed changes in the lungs typical of death from anoxia.

Two mice tested under the same conditions and at the same pressure for 8 hours were decompressed in 6 hours. They appeared normally active while under pressure in comparison with their littermates outside the chamber and have subsequently shown no signs of deterioration.

Four mice were then tested at 62 atm. After 3 hours at this pressure the chamber was ventilated with a mixture of 0.6 percent oxygen and 99.4 percent helium. One hour later one of the animals had stopped breathing and the other three appeared lethargic. Oxygen was added and the three remaining mice survived 6 hours at 62 atm and suffered no ill effects after decompression. A gas sample taken from the chamber immediately after the one animal was observed to be dead and before oxygen was added was analyzed (*11*) and found to contain 0.2 percent oxygen and no measurable carbon dioxide. This oxygen tension was equal to 0.12 atm and anoxia was the most probable cause of death.

Table 1. The effects of exposing mice to hyperbaric helium-oxygen atmospheres. The depth of sea water equivalent to 42 atm is 396 m; 63 atm is equivalent to 615 m, and 92 atm to 914 m.

No. of animals	Pressure (atm)	Duration of exposure (hr)	Decompression time (hr)
0.7 percent oxygen, 215 mm-Hg			
2	42	13.66	20
0.6 percent oxygen, 281 mm-Hg			
2	62	15	
2	62	6	3
4	62	6	3
2	62	2	3
4	62	2	3
4	62	2	3
0.65 percent oxygen, 480 mm-Hg			
3	92	1	4.66

Ten mice were subsequently subjected to a pressure of 62 atm under similar conditions for 2 hours. In nine of the ten mice there were no immediate or delayed adverse physiological effects. The weight of each animal before the experiment remained the same for an observation period of 2 weeks after decompression.

The single mouse affected convulsed during the final stages of decompression. Seizures were relieved by increasing the pressure from 17 atm to 20.4 atm, but recurred when pressure was again lowered to 13.6 atm. After decompression the mouse exhibited a delayed righting reflex and paralysis of both hind legs. It died several hours later despite recompression therapy. This failure appeared to be the result of inadequate decompression, since only half the time previously taken was used in this attempt at continuous decompression. The other three mice in the same experiment tolerated the relatively rapid decompression with no signs of immediate or delayed decompression sickness.

In the final experiment, three mice were exposed to a pressure of 92 atm, equivalent to a depth of 914 m of sea water. The mice were placed in the chamber which was purged with a mixture of 20 percent oxygen and 80 percent helium, and the pressure raised to 3 atm (gauge) with this mixture. The oxygen tension was 0.6 atm. The pressure was then raised to 92 atm absolute with pure helium. Two minutes after attaining this pressure, one mouse convulsed and died. The two remaining were active during the 1-hour exposure period. Decompression was accomplished in 4 hours and 40 minutes. In the two survivors there were no residual effects. The results of the experiments are summarized in Table 1.

The decompression schedules which followed exposure to pressures equivalent to depths of 610 and 914 m are of interest. The decompression was begun by lowering the pressure by 4 atm ($\triangle P$ atm). After 5 minutes it was estimated that the $\triangle P$ had decreased 2 atm. Hence, throughout the remainder of decompression, the pressure was reduced by 2 atm every 5 minutes. At 2 atm a final stop was made for a period of 15 minutes. Decompression from 60 atm was achieved satisfactorily in 3 hours, regardless of how long the mice were exposed to the abnormal pressure.

The chamber was ventilated periodically with premixed oxygen and helium to rid the chamber of excess carbon dioxide and urinary ammonia, and to increase oxygen tension as the pressure was decreased.

We were not aware of previous tests in which mammals have been maintained for many hours in apparently good condition at pressures in the range of 60 to 90 atm. Extensive test on lower animals may make it possible for men to find ways of living and performing useful work at depths of 610 or 914 m. Graded exposures of man may well extend practical diving depths from 152 to 914 m or more.

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