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Survival of Mice in Absence of Inert Gas.* (25983)

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It has been generally assumed, for lack of evidence, that the nitrogen gas of the earth's atmosphere plays no role as a respired gas in the metabolic function of higher animals. However, since N_2 at pressures above 2 atmospheres begins to interfere with the normal behavior of the nervous system(1), it appears justified to ask whether or not it may at .79 atmosphere exert some effect on animal systems. The answer to this question is to be sought by looking for any biological changes in animals maintained in absence of N_2 gas. As a means of freeing the environment of N_2 gas, substitution of another inert gas for N_2 is not satisfactory since the other gas may of itself modify the results. On the other hand an atmosphere of pure O_2 at normal pressure is unsuitable since this will prove lethal in a matter of a few days. To test the possible long-term effects of absence of any inert gas upon the function of animals it is therefore necessary to use an atmosphere of pure oxygen but at reduced pressure to avoid oxygen toxicity. Our experiments indicate that mice will survive for long periods of time in oxygen when its pressure is maintained at 197 mm Hg. This is the level which provides the same inspired O_2 tension (150 mm Hg at BTPS) as breathing air at 1 atmosphere. Furthermore, 197 mm Hg of pure O_2 is the atmosphere which provides for a satellite cabin in space the lowest pressure differential across the walls compatible with a normal inspired O_2 tension within.

Method. The major problem is a mechanical one, that of maintaining animals for long periods of time in an atmosphere of O_2 at a reduced pressure. To avoid introduction of N_2 gas from the air into the chamber, the arrangements must render access to the animals unnecessary during the course of an experiment lasting many weeks. Chamber pressure and oxygen ventilation must be automatically maintained within narrow limits by reliable means and temperature and humidity must be kept within the comfortable range. In addition food and water must be provided and excreta conveniently accommodated. Since it took many months of trial and error to arrive at a satisfactory solution, a brief description of our arrangement follows. With the exception of the watering device, the gas sampling system and the water vapor trap,† all other parts are commercially available. The apparatus is schematically represented in Fig. 1. A small cast-aluminum chamber‡ (10

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† An acrylic resin chamber sealed around the cooling coil of a small vapor absorption type refrigeration unit (Astral Refrigerette).

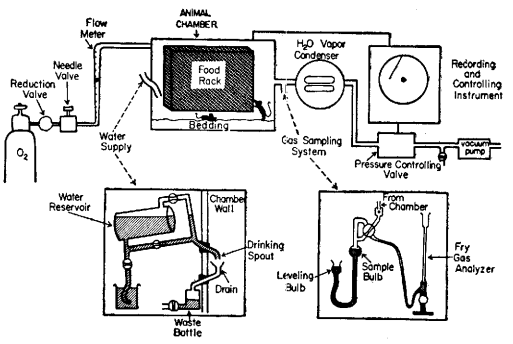

FIG. 1. Altitude chamber system for long-term maintenance of mice in oxygen at reduced pressure. Chamber is continuously flushed with O_2, flow regulated by needle valve. Controlling valve in outflow line is operated by recording pressure controller to maintain chamber pressure constant. Water supply and gas sampling systems shown in detail.

x 10 x 18″ inside) with a windowed door (7 x 12″) housed the animals. The chamber was continuously flushed with a stream of "medical" O_2. A reducing valve was followed by a needle valve across which the gas pressure dropped from above to below atmospheric pressure. Oxygen flow into the chamber was controlled by the needle valve and continuously monitored by a rotameter. To maintain CO_2 tension in the chamber below 5 mm Hg the required flow was calculated from the alveolar ventilation equation, putting in, for "CO_2 production," that of all the mice in the chamber, and for "alveolar ventilation," the oxygen flow. In both chamber and alveoli the steady-state gas concentrations are independent of volume of the ventilated space. Transcribing the alveolar ventilation equation (2) to the ambient temperature and pressure conditions of our experiment, one obtains this relation: chamber CO_2 (mm Hg) is equal to .83 x CO_2 output of all mice (ml, STPD, per min.) divided by oxygen flow (1, ATPD, per min.). Thus, for example, if CO_2 output of 6 mice is equal to 10 ml/min., then a flow of 2 liters/min. of O_2 at the reduced pressure will maintain (10 x .83)/2 or 4 mm P_{CO_2} in the chamber. Periodic analysis of the chamber gas confirmed these predictions. CO_2 concentration was never observed to exceed 5 mm P_{CO_2} and was usually lower. Chamber gas samples were obtained periodically by the device shown in Fig. 1. A small sample of gas was aspirated from a T tube in the exhaust line by means of a mercury reservoir. This gas could then be compressed after turning a stopcock and shunted directly through a connecting tube into a Fry gas analyzer for CO_2, O_2 and N_2 analysis. This procedure not only allowed one to monitor CO_2 concentrations but also to detect any inward leaks of N_2 which might occur during the weeks of uninterrupted experiments. The inflowing gas was continuously removed from the chamber by a vacuum pump after first passing over a refrigerated vapor trap to remove the moisture. The pressure was maintained by a servo-operated pressure controlling valve§ and was continuously recorded throughout the whole experiment. Thus any failure to maintain pressure could be easily detected. The sensitivity of the pressure controlling valve was ± 2 mm Hg. The temperature of the chamber was maintained between 24° and 27° by a thermostat operated heater. The mice were bedded on 2″ of Sterolit.‖ This powdery material absorbed all the waste products and prevented their further decomposition. Food was provided in the form of Purina Fox Chow or Old Guilford mouse pellets. These were placed in a closed wire mesh container which held enough food for duration of experiment. Water was supplied from the outside by means of a system illustrated in Fig. 1. This system was found to be necessary since simpler devices failed to work adequately under reduced pressure. By proper manipulation of stopcocks additional water could easily be supplied at any time. At start of experiment the chamber was flushed with 100% O_2 which the animals breathed for about 10 minutes. The chamber was then evacuated until final pressure was reached in about 15 minutes. All animals used were Swiss mice with the exception of four C57

‡ The authors are greatly indebted to the Dept. of Physiology, School of Aviation Medicine, Randolph Air Force Base, for the loan of this chamber.

§ Motosteel Evenaction valve, actuated by a recording Fulscope absolute pressure controller (Taylor Instrument Co., Rochester, N. Y.).

‖ "Sterolit", Minerals & Chemicals Corp. of America, Menlo Park, N. J.

TABLE I. Accident-Free Sojourns of Mice in 197 mm Pure O_2 Atmosphere.

Exp. #	Mice		Days in chamber
1	6	adult male	55
3	6	2-mo "	18
8	6	adult female	12
	11	2-14 day fetus	"
9	2	lactating female	19
	18	suckling young	"
10	2	adult female	51
	6	fetus—young (conception to 27 day)	48
	1	adult male	22
	2	" female	"
	14	fetus—young (conception to 1 day)	"
	4	C 57 black female	"
	4	C 57 Swiss fetus	5

black females used in the breeding experiment.

Results. In 5 different experiments (summarized in Table I) mice were maintained in pure O_2 at 197 mm Hg total pressure for a total of 1308 mouse-days (53 different mice) with no apparent ill effects. In several other experiments a particular type of lethal accident occurred. This will be described below.

Young mice passed all the stages from conception to birth to weaning and adolescence in the chamber in an apparently normal fashion. Microscopic examination of 14-day fetuses, and of the eyes of those conceived and born in the chamber, revealed no abnormalities.

Of the adult mice that were kept in the chamber one group of 6 males after 55 days appeared normal as revealed by histological sections of heart, kidney, spleen, liver, lungs, thyroid, and eyes. Other groups remained apparently healthy and carried on the functions of mating, fertilization and implantation of ova, gestation, bearing and suckling of young in a normal fashion. The longest uninterrupted stay in the chamber was 51 days, by 2 females which conceived, bore and raised young there.

In the breeding experiment one male put into the chamber with 8 females (4 Swiss, 4 C57 black) impregnated the 4 Swiss females within the first 3 days and at least 2 of the C57 females about 2 weeks later. The 4 Swiss females bore their young in the chamber. The first 2 litters were removed from the chamber, along with the mothers, one day after birth. All 14 of these young removed from the chamber survived and developed normally for at least 8 weeks. The second 2 litters were left undisturbed in the chamber, where the 2 mothers ate all but 6 of the young within the first day or two. The surviving 6 were then raised to 27 days of age in the chamber before ever being exposed to normal air. The retinas of young conceived and born in the chamber and of the 2 females which stayed there 51 days were later given careful histological examination, which showed them to be apparently normal.

The one adverse effect observed in these experiments was the death of some mice within the first 48 hours of exposure to the reduced pressure. This phenomenon appeared to be a result of the decompression itself, since it never occurred after the first 48 hours. In a total of 115 mouse decompressions 20 mice died. Typically the mice that died this way became relatively inactive immediately after the reduction in pressure, and appeared to sleep most of the time until death. In the late stage they tended to sit hunched up, with fur erect, breathing in large gasps. On autopsy their lungs were found to be almost completely atelectasized. Of particular interest were four young mice which died. These animals were conceived, born and raised in the chamber in the absence of inert gas. They were finally brought out into normal air at 27 days of age. After 2 hours of breathing air for the first time in their lives they were again placed in the chamber and returned to their original environment of O_2. They succumbed within 24 hours.

Discussion. The only statement that can be made on the basis of these observations is that life and reproduction are possible for a mammal in the absence of all but a small fraction of the inert gas that is present in air. This life appears, on gross observation at least, to be essentially normal with the exception that fatalities may occur immediately following decompression.

It seems likely that these fatalities are due to collapse of alveoli following minute ob-

structions (mucous) of the smallest airway. Recent evidence(3,4) suggests the existence of a normal tendency of some alveoli to collapse, a tendency that is ordinarily kept in check by reexpansion of collapsed alveoli upon deep inspiration (sigh). It has been shown (5) that under 1 atmosphere of pressure rate of collapse following obstruction is 60 times faster on pure O_2 breathing than on air breathing. When pure O_2 is breathed the collapse of a blocked-off alveolus is determined only by removal of O_2 by the perfusing blood. (CO_2 removal is secondary to O_2 removal, which by raising concentration of CO_2 in the remaining gas, reverses the normal CO_2 gradient.) Thus the time for collapse, assuming perfusion rate to be constant, should be approximately proportional to number of molecules of O_2 present. Therefore, the effect of lowering total pressure at which pure O_2 is breathed will, by lowering absolute volume-concentration of O_2, speed up the collapse of obstructed alveoli, in accordance with the following relation:

$$\text{Collapse time} = k\,P_{A_{O_2}} = k\,(P_B - P_{A_{H_2O}} - P_{A_{CO_2}}).$$

In the case of $P_B = 197$ mm Hg, therefore, $P_{A_{H_2O}}$ and $P_{A_{CO_2}}$ being assumed to remain normal at 47 and 40 mm Hg respectively, the collapse time should be approximately (197-87)/(760-87) or 1/6 of that for $P_B = 1$ atmosphere. This then would suggest that under our experimental conditions rate of collapse of a blocked alveolus is increased about 360 times above the value for breathing air at 1 atmosphere. It is conceivable that at this rate the tendency to collapse may in some circumstances prove too much for the forces which normally operate to restore the alveoli. However, this account does not explain why the deaths observed should all occur during the first 48 hours in the chamber. It may be speculated that all the mice that died were suffering from some condition in which increased bronchiolar secretions rendered them more prone to airway obstruction and atelectasis.

Conclusion. It is concluded that an inert gas such as N_2 is not necessary for maintenance of normal metabolic processes. However, the proneness for development of pulmonary atelectasis is exaggerated in absence of an inert gas such as N_2.

Summary. 1) Mice were maintained for 51 days in an atmosphere of oxygen at total pressure of 197 mm Hg. This provides a normal inspired O_2 tension. CO_2 and N_2 pressures in the ambient atmosphere did not exceed 5 mm Hg for either gas. 2) Under these conditions animals usually did not encounter any difficulties. Several litters were born and in one case young were conceived, born and raised in virtual absence of inert gas. However, in several cases animals died within 48 hours after being subjected to this environment. Once past this period only mechanical failure of the apparatus terminated these experiments. It is suggested that pulmonary atelectasis was responsible for these early deaths.

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