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Received March 2, 1965. P.S.E.B.M., 1965, v119.

Residual Effects of a Single Exposure to Hyperbaric Oxygen in Mice.* (30301)

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The use of hyperbaric oxygenation is gaining acceptance as therapy in a number of medical and surgical diseases(1). However, this therapy often requires several treatments for a number of days, and there is evidence that repeated exposures to high pressures of oxygen might sensitize the central nervous system to oxygen toxicity. Almeida reported in 1934 that rats exposed to oxygen at 6 atmospheres of pressure (absolute) until they convulsed, in subsequent exposures would convulse within ⅓ to ¼ the initial time(2). Bean reported similar results(3). Fenn observed that daily exposures of the fruit fly to 1 atm. of O_2 for 6 hours every day, will decrease the life span by 10 to 15%(4).

The present experiments were planned to determine if repeated exposure to hyperbaric O_2 in the range used clinically would sensitize mice to convulsions. The length of exposure used was selected so as not to produce any convulsions during the first pressurization.

Method. Male Paris mice weighing between 25 to 30 g were exposed to hyperbaric oxygen in a small, transparent, lucite pressure chamber. The floor of the chamber was covered with a carbon dioxide absorbant (baralyme), and before pressurization the chamber was flushed with 100% O_2 for 5 minutes and then brought to the desired pressure in 4 to 5 minutes. Gas flows were adjusted to maintain a constant pressure in the chamber while allowing some O_2 to continuously escape. The mice were constantly observed throughout pressurization and the time of onset of convulsions was recorded. The animals were subjected to a varied number of exposures at either 3 atmospheres pressure absolute for 15 minutes or 4 atm. press. absolute for 5 minutes. When more than one exposure per day was given, an hour was allowed to elapse between experiments. Between exposures the animals were given food and water *ad lib.*

In the first series of experiments, 144 mice were exposed to 3 atm. press. for 5 days: 48 mice were exposed once a day; 24 twice a day; 24 four times a day and 48 once every other day (3 exposures). In the second series, 288 mice divided into 6 groups were exposed to 3 atm. press. and during the following 6 days, one of the groups was given an additional exposure. The delay between exposures thus varied from one to 6 days. In the third series of experiments, 144 mice were exposed to 4 atm. press. for 5 days: 24 mice were exposed once a day; 48 twice a day; 24 four

* This work was supported in part by N.I.H. grants RIO-CA-06601-03 and GM-09069-03.

TABLE I. Incidence of Convulsions (%) in Mice After Successive 15 Min Exposures to O_2 at 3 Atmospheres Absolute.

Exposures per day	No. of mice	Day 1				Day 2				Day 3				Day 4				Day 5			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
4	24	0	83	88	83	96	46	42	58	100	75	79	100	100	75	79	70	100	88	67	70
2	24	0	29			100	83			100	100			83	88			96	92		
1	48	0				56				72				73				66			
1 on alt. days	48	0								94								100			

TABLE II. Incidence of Convulsions (%) in Mice After Successive 5 Min Exposures to O_2 at 4 Atmospheres Absolute.

Exposures per day	No. of mice	Day 1				Day 2				Day 3				Day 4				Day 5			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
4	24	0	33	33	29	27	41	50	45	64	36	59	36	55	73	45	64	45	32	50	55
2	48	83	38			45	55			57	68			74	50			80	72		
1	24	0				33				67				58				54			
1 on alt. days	48	0								63								69			

times a day; and 48 were given one exposure every other day (a total of 3 exposures).

At the end of each experiment the animals were sacrificed and a gross examination was made of the lungs. The testicles were removed, fixed in 10% formalin, and microscopic slides prepared. Any mice that died during the experiments were autopsied and their lungs carefully examined.

In the first series of experiments at 3 atm. press. none of the mice convulsed on the first exposure. On the second exposure from 29 to 94% convulsed. The group of mice subjected to 4 exposures a day did not have a higher percentage of convulsions (Table I). Average time of onset of convulsions after full pressure was attained ranged from 4.2 to 10.8 minutes and could not be related to the number of exposures. For individual animals, the onset of convulsions following the initial pressurization also varied widely, ranging from 0.5 to 14.5 minutes.

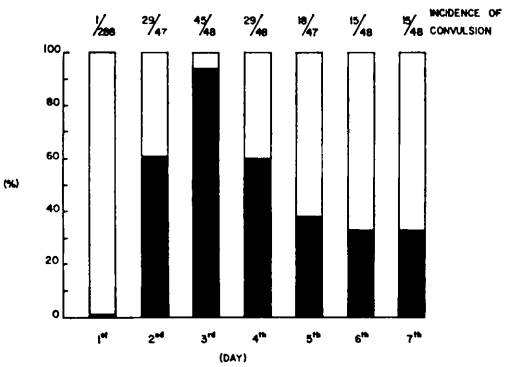

FIG. 1. Incidence of convulsions in mice following 2 exposures to 3 atmospheres of O_2 for 5 min (second exposure given 1 to 6 days after the first).

Fig. 1 summarizes results of the second series of experiments at 3 atm. press. with each of 6 groups receiving one additional exposure at varying intervals. One mouse convulsed on initial pressurization and 2 suffered mechanical trauma during the first exposure and died within 24 hours. On autopsy these 2 animals had normal lungs. On second exposure between 32% and 94% of the mice convulsed, the lowest percentages occurring when exposure was 5 days following the first and the highest incidence when the 2nd exposure was 2 days later.

Table II summarizes results obtained when mice were exposed to oxygen at 4 atm. press. for 5 minutes. Of a total of 144 animals, 2 convulsed during the first pressurization (1.3%). None of the mice pressurized once a day or on alternate days died. In the group receiving 2 exposures a day, a mouse died on the second day and another on the fourth day (4.2%). Two of 24 mice (8.3%) exposed 4 times a day died after the first day. On autopsy all of these mice exhibited the classic "liverlike" lungs observed in oxygen toxicity. Convulsions occurred at any time from the beginning of full pressurization to the start of decompression. The average time of onset of convulsions ranged from 2.0 to 4.5 minutes.

Microscopic examinations of the testes showed no testicular degeneration in any of the mice exposed to either 3 or 4 atm. press. In all the experiments on the second exposure to hyperbaric oxygen, convulsions occurred in a significant number of mice although they had no apparent reaction to an earlier identical first exposure. This effect still persisted 6 days after the initial pressurization. Aside from an increased mortality rate in the groups of mice exposed to 4 atm. press., there was no apparent pattern of cumulative toxic symptoms or tolerance to hyperbaric oxygen. Some of the animals did not convulse after a second exposure, indicating a wide range of individual tolerance to hyperbaric O_2, a finding which is well documented(3). Administration of chlorpromazine (20 mg/kg intraperitoneally) to the mice before the first exposure did not alter the incidence of convulsions following a second exposure.

The results of the present experiments provide additional evidence that there is a sensitization of the central nervous system of rodents to the toxic effects of hyperbaric oxygen. The mechanism of this phenomenon and the extent of its presence in man remain to be established.

Summary. Mice that did not convulse after an initial exposure to oxygen at 3 or 4 atmospheres of pressure (absolute), convulsed in significant numbers after a second exposure. This residual effect of "hyperbaric oxygen" lasted as long as 6 days.

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Received April 26, 1965. P.S.E.B.M., 1965, v119.

Role of Interferon and Partial Immunity in Influenza Virus Infection of Mouse Lung. (30302)

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Isaacs and Hitchcock(1) reported that a virus inhibitor similar or identical with interferon (ITF), produced in the lungs of nonimmune mice infected with a sublethal dose of influenza virus (PR8), promoted recovery. The presence of ITF in the lung prior to the appearance of neutralizing (NT) antibody in the serum supported this view. Loosli *et al*(2) showed that mice immunized with influenza virus (PR8) and exposed to a lethal aerosol of homologous virus became moderately ill but survived, whereas nonimmune controls died. In the present study, these findings were reexamined and extended by studying simultaneously the effect of ITF and NT antibody on influenza virus (PR8) infection of partially immunized mice.

Materials and methods. Viruses. The PR8 strain of influenza A virus was obtained through the courtesy of Merck, Sharp and Dohme Laboratories, West Point, Pa. The virus had received 128 allantoic passages in chick embryos and 4 mouse lung passages to produce a stock pool having a lethal mouse