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Suppression of Hyperoxic Convulsions by Nitrogen at High Pressure.* (20412)

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Present information regarding the effect of breathing air at high pressures indicates that disturbances arise from the action of either oxygen or nitrogen on the nervous system(1). It is the purpose of this paper to present evidence that the depressant effect of nitrogen in this respect is actually antagonistic to the excitant action of oxygen when both gases are at increased pressure.

Method. Adult male and female mice were used in the experiments which were carried out in a steel chamber equipped with a window and large enough to accommodate 8 animals. Each mouse was isolated in an open ended glass jar standing upright on coarse wire mesh over soda lime. Approximately 2 minutes were required to fill the vessel to the desired gas pressure and the time for the appearance of the first convulsion in each mouse was recorded after the pressure of oxygen reached 90 psi.

Results. Usually the first indication of central nervous excitation in mice exposed to oxygen at high pressure is the presence of tail erection (Straub's sign) occurring at any time in the preconvulsive period. During the convulsive seizures clonic movements of the limbs are produced with such violence that the animal is propelled upwards against the restraining jar with considerable force. In males this is accompanied by ejaculation and in both sexes by urination. When the convulsion subsides the animal appears to be in no distress until the third or fourth seizure when obvious depression supervenes characterized by gasping-retching movements. The variation in convulsive behavior in mice after exposure to 90 psi of oxygen can be seen by reference to Fig. 1 (top) where the percent of animals involved in seizures is compared with the duration of their preconvulsive period. Convulsive behavior in mice first exposed to helium at 400 psi for 5 minutes as a control before the addition of 90 psi of oxygen is shown in Fig. 1 (middle). In both cases all animals displayed several seizures, as described above, before termination from respiratory failure. In mice first exposed to 400 psi of nitrogen for 5 minutes, before the subsequent addition of 90 psi of oxygen into the chamber, convulsions were produced in only 23 percent of the animals, and their median preconvulsive period was increased 44 percent beyond that of the control series in oxygen alone. Fig. 1 (bottom).

There is considerable difference between

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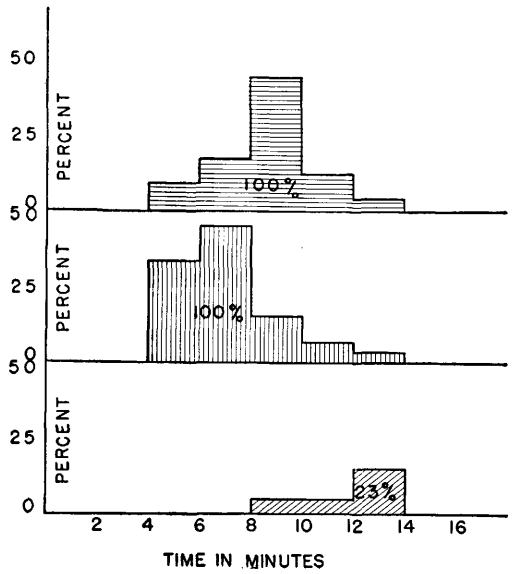

FIG. 1. Onset of convulsive activity with time in mice: Ordinate: % of total animals exposed. Abscissa: Time for appearance of first seizure. 25°C *Top:* At 90 psi of oxygen (36 animals). *Middle:* At 90 psi of added oxygen 5 min. after the mice were exposed to 400 psi of helium (33 animals). *Bottom:* At 90 psi of oxygen added 5 min. after the mice were exposed to 400 psi of nitrogen (42 animals).

convulsions produced in oxygen or helium-oxygen and those in nitrogen-oxygen. In the former case clonic discharges of the limbs become wild and rampant while in the latter instance the limb movements were restricted to twitches or tremors, but are indicated as convulsions in Fig. 1. The remaining 77% of the mice showed no recognizable convulsive activity whatsoever. In addition to the effects just described ejaculation and urination were never as copious as in the control animals. It should be emphasized however that the nitrogen treatment did not increase the survival or eliminate the pulmonary damage seen in the control group.

Discussion. One may note a shorter median preconvulsive period with helium-oxygen as compared with that for oxygen alone. The helium mixture due to its high thermal conductivity at this pressure (5.7 times that of water) may increase the susceptibility of the mice by lowering body temperature and initially increasing oxygen consumption. The latter condition is believed to potentiate oxygen toxicity according to the studies of Grossman and Penrod(2) and others(1). In measurements made in this laboratory an initial decline in rectal temperature amounting to .33°C/minute has been observed over a 20-minute period when mice are exposed to 400 psi of helium at 25°C. Oxygen consumption studies at this pressure and temperature however have not been made.

Bert(3) observed in dogs that tonic seizures caused by oxygen at high pressure were alleviated by chloroform and he therefore attributed them to exaggerated activity in the central nervous system. He noted a similarity between this action of oxygen and the symptoms of strychnine poisoning. Shilling and Adams(4) reported that rats given anesthetic doses of barbital or pentobarbital did not develop convulsive activity when exposed to OHP. Marks(5) has since demonstrated that several other CNS depressant drugs are capable of delaying the onset of hyperoxic seizures in mice and cats.

The depressant action of nitrogen at increased pressure reported by others(6,7) has recently been found to protect mice from electroshock seizures at pressures ranging from 220-400 psi(8). In these experiments it was observed that after a saturation period of 5 minutes the degree of protection afforded was proportional to the nitrogen pressure. It would seem therefore, that pressures beyond 400 psi of this gas would reduce still further the percentage of animals in our experiments showing hyperoxic CNS manifestations while practically no anticonvulsant action could be expected below 220 psi. However, it was of considerable interest that with the use of nitrogen alone, at this pressure, mice exhibit only slight depression in voluntary movements and are apparently able to right themselves or stand erect.

Summary. The time of onset and severity of convulsions was observed in 3 groups of mice exposed to 90 psi of oxygen, the first receiving the oxygen alone, the second 400 psi of helium plus oxygen and the third 400 psi of nitrogen plus oxygen. All the animals in groups one and two convulsed within 15 minutes of exposure to oxygen at this pressure but in the third group much less violent seizures appeared in only 23% of the mice and were delayed 44% in time of onset. Voluntary

movements and postural reflexes were not seriously disturbed by this pressure of nitrogen.

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Erythropoietic Stimulation Induced by "Anemic" Serum.* (20413)

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Carnot and Deflandre(1) suggested that red cell production is governed by a factor present in blood serum. Since then numerous investigators have attempted to demonstrate this factor in serum and plasma from animals rendered anemic by bleeding or hypoxemic by exposure to air with a low oxygen tension(2). All the results were either negative or so equivocal that there has been a general refusal to accept the hypothesis of a humoral regulation of red cell production. Krumdiech(3) reported that 20 ml of serum from anemic rabbits induced a striking reticulocytosis when injected into normal rabbits. These results were not confirmed by Toha *et al.*(4) who subsequently injected 20 ml of "anemic" rabbit serum once a day for 3 days followed by 10 ml a day for 15 days into normal rabbits and were unable to demonstrate more than a slight rise in the number of reticulocytes. Erslev(5) was likewise unable to demonstrate a significant rise in reticulocytes when similar amounts of "anemic" plasma were used. However, when 200 ml of "anemic" rabbit plasma was administered to normal rabbits during a 4-day period a significant increase in the number of reticulocytes was found. The bone marrow showed a concomitant rise in the relative number of normoblasts and when 650 ml of "anemic" plasma was given during a period of 18 days there was a definite rise in hematocrit values and red blood cell count. These experiments showed that in rabbits red cell production can be stimulated by a humoral plasma factor. Reissman's(6) study with parabiotic rats tends to indicate that a similar factor exists in plasma from anemic rats.

Before further investigation was carried out into the nature and production of this factor it was felt advisable to demonstrate its presence in an animal species more closely related to humans than rabbit or rat. Macaca mulatta monkeys were selected for this purpose.

Materials and methods. Adult Macaca mulatta monkeys were used as donors and recipients. Three monkeys were made anemic by bleeding. They were bled from the femoral artery or vein after the animals had been anesthetized with 2.5 ml 6.0% nembutal given intravenously. Two or 3 times a week 80-100 ml of blood was withdrawn and "anemic" serum was obtained and pooled after the hemoglobin had been reduced to below 7 g/100 ml. Part of the pooled serum was kept for 2 weeks at 4°C while the remainder was frozen in a dry ice box. Serum from normal monkeys was ob-

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