Hypoxia Tolerance of Rats after Adaptation to Hypercapnia* (34097)

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In a closed environment, such as a spacecraft, numerous changes in the gaseous environment could occur as a result of a malfunction in the control system. Two possible consequences might be a decrease in oxygen partial pressure and an increase in CO_2 partial pressure. The buildup of CO_2 would probably be slow with a gradual adaptation to hypercapnia by the occupants. Hypoxia, on the other hand, could happen quickly—as with the loss of cabin pressure. If the two situations occur sequentially, physiological effects are different than when they occur separately. In other words, how might a man's tolerance to a sudden hypoxic episode be altered if he had first become adapted to a high CO_2 level?

A study of this type is of interest not only for practical reasons but because it involves basic questions concerning the control of respiration. Fencl *et al.* (1) have strongly suggested that resting ventilation is a single function of $[H^+]$ concentration in the cerebral interstitial fluid. During hypercapnia the partial pressure of CO_2 is increased in all body fluids, resulting in greater amounts of dissolved CO_2. Conversion of this CO_2 into carbonic acid increases the $[H^+]$ concentration. $[HCO_3^-]$ level in the cerebral interstitial fluid which bathes $[H^+]$-sensitive respiratory receptors is increased in order to bring $[H^+]$ back to normal (2). If a CO_2-adapted animal is then suddenly exposed to hypoxia and CO_2 is removed, cerebral interstitial $[H^+]$ should fall rapidly and this would inhibit the normal increase in respiration seen with hypoxia thus decreasing hypoxic tolerance. This hypothesis was tested on rats in the experiments to be described.

Methods. Young adult Wistar CD strain male rats were used as experimental animals. Pairs were housed in clear plastic cages. Pine shavings were used for bedding and changed twice weekly. Purina Laboratory Chow and water were provided *ad libitum*.

The rats were tested for time of useful consciousness (TUC) in an altitude chamber having a volume of about 300 ft^3. They were placed in a box 14 × 17 × 9 in., that was completely screened except for one side which contained a metal plate along the floor (Fig. 1). This plate was electrified with a variable-voltage power supply having a maxium voltage of 34 V at 5 A. In addition the box was tilted at a 32-deg angle so the rats had to cling to the screen to keep from sliding over the electrified plate. The entire box was raised above the floor to prevent the rats from jumping out.

The rats were tested in pairs. They were placed in the box, the grid was electrified, and the chamber evacuated to 632 torr[1]. The chamber was held here for a few seconds in order to obtain an accurate starting time. It was then evacuated to a pressure corresponding to an altitude of 30,000 feet, 226 torr, in a time as near to 2.5 min as possible. It was held there until both rats had fallen from the box and then was returned to ground level.

* The research reported in this paper was conducted by personnel of the Physiology Divison, Respiratory Physiology Branch, USAF School of Aerospace Medicine, Aerospace Medical Division (AFSC), United States Air Force, Brooks, AFB, Texas. Further reproduction is authorized to satisfy the needs of the U.S. Government. The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences—National Research Council.

[1] torr = mm Hg.

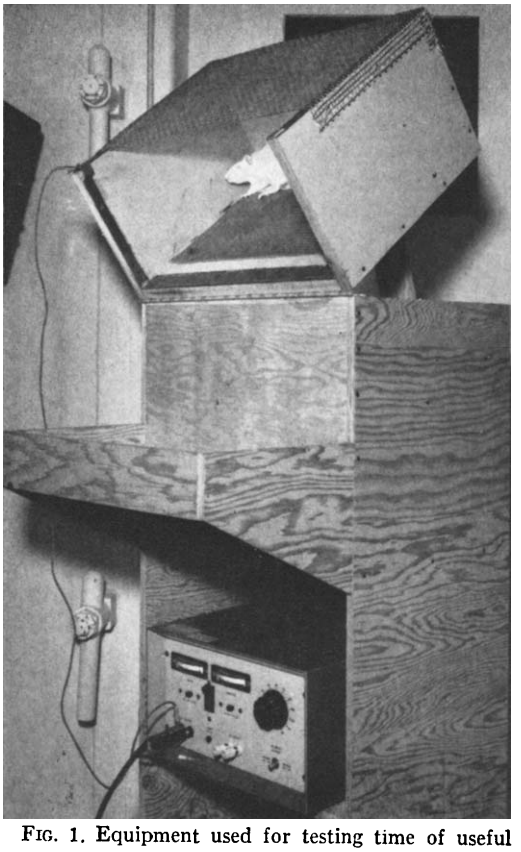

FIG. 1. Equipment used for testing time of useful consciousness.

The rats were conditioned to CO_2 in separate chambers of similar size. Total pressure, CO_2, oxygen, and relative humidity were automatically controlled and continuously monitored. Total pressure was held at 760 torr, slightly above ambient, oxygen 21%, relative humidity 40–50%, temperature 75°F, and CO_2 90 torr. Carbon dioxide was flushed in directly from a cylinder until the desired level was attained and the automatic controls then were set.

The rats were tested for TUC, initially, to obtain normal values. Each, therefore, served as its own control. They were then allowed a month to recover. After the recovery the rats were exposed to hypercapnia continuously for 3–4 days until tested again for TUC. The TUC test was begun within 5 min after removal from the high CO_2 environment.

A record was made of the time required to evacuate the chamber from 632 to 226 torr, and the time after reaching 226 torr until the rats fell from the box. The latter time was our criteria for TUC. Differences between control and experimental TUC were compared with the aid of a paired t test (3).

Results. The results are shown in Table I. Of an original 30 animals tested for TUC, comparisons are shown for 23. Three rats died during control tests. The cause of death appeared to be the result of tetany which inhibited respiration. Two CO_2-adapted rats suffered from tetany during decompression to 226 torr. Two others jumped from the testing box prior to becoming unconscious.

Except for the two rats that jumped from the box, the animals clung to the screen as long as possible. As they became progressively hypoxic the rats would slide down the box until they received a shock and would then climb up again. After sliding and receiving

TABLE I. Time of Useful Consciousness of Rats at 226 Torr before and after Adaptation to Hypercapnia, in Minutes.

Rat number	Control	Experimental
1	1.00	16.46
2	0.83	4.05
3	12.65	1.52
4	15.15	1.01
5	2.22	1.45
6	3.99	16.30
7	23.92	1.80
8	86.15	1.80
9	11.12	2.00
10	12.43	1.55
11	22.50	1.01
12	17.20	1.45
13	29.90	1.35
14	65.48	27.55
15	3.37	2.55
16	14.07	3.90
17	19.03	17.99
18	10.75	2.90
19	0.93	0.17
20	20.00	1.63
21	2.78	1.25
22	1.63	1.25
23	27.42	0.32
Average	17.59	4.83

$t = 3.02$; $t < .01 = 2.819$.

four or five shocks the rat could no longer hold on and would slide over the electrified grid into a container about 2 ft below. They would then lie quietly with their eyes closed and make no further attempts to move.

The average TUC for the 23 comparisons was 4.83 ± 1.52 min after CO_2 adaptation and 17.59 ± 4.31 min during the control tests before the animals were adapted to CO_2. A paired t test indicated that these differences were highly significant ($p<.01$).

Discussion. During acute exposure to hypoxia, chemoreceptor reflexes from the carotid and aortic bodies stimulate ventilation increasingly with decreasing arterial Po_2 levels. As pulmonary ventilation increases, arterial Pco_2 levels progressively drop. Dissolved CO_2 is in equilibrium with carbonic acid and, consequently, $[H^+]$ levels also decrease (4). Since CO_2 is freely diffusible throughout body tissues, any decrease in arterial Pco_2 will also result in a decreased cerebral spinal fluid (CSF) Pco_2 and a concomitant decrease in CSF $[H^+]$ concentration. As a result, the central respiratory chemoreceptors are less active and thus tend to inhibit the effectiveness of the peripheral chemoreceptor respiratory drive (5). In acute hypoxia, the respiratory minute volume seldom increases as much as 100%. However, if sufficient CO_2 is added to the inspired air under these hypoxic conditions to maintain a normal Pa_{CO_2} pulmonary ventilation can increase as much as seven times normal (6). This indicates that hypoxia is actually a strong respiratory stimulus if the inhibitory effect of alkalosis is removed.

In a chronic hypoxic environment, CSF $[Hco_3^-]$ decreases during the course of 1–2 days (5, 7). This decrease in buffering capacity, which is an important part of the adaptation process to hypoxia, permits pulmonary ventilation to increase without causing a significantly large decrease in SFC $[H^+]$ levels. Conversely, during adaptation to chronic hypercapnia, CSF $[Hco_3^-]$ levels increase to offset the increased acidosis resulting from the higher levels of dissolved CO_2 (8).

If an animal is adapted to chronic hypercapnia and then suddenly exposed to a CO_2-free environment, the increased buffering of the CSF will result in a lower $[H^+]$ concentration for a given level of respiration than in the CO_2-unadapted animal. Thus, the inactivity of the central chemoreceptors in the adapted animal should act as a strong block toward any peripheral chemoreceptor drive and should be particularly noticeable during acute exposure to hypoxia. Our data suggest that this was indeed the case and that resistance to hypoxia was greatly reduced after adaptation to chronic hypercapnia.

Summary. Thirty rats were tested for time of useful consciousness (TUC) by recording the time they could cling to a steeply sloping surface after decompression to a barometric pressure corresponding to 30,000 ft altitude (226 torr). After testing, the rats were returned to ground level conditions for 1 month. They were then exposed continuously for 3–4 days to an environment containing a partial pressure of 90 torr CO_2 and tested for TUC again immediately after removal from the high CO_2. TUC decreased from 17.6 min to 4.8 min ($p<.01$) after adaptation to hypercapnia. These data support present theories concerning the role of CO_2 in the regulation of respiration.

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