Interaction of Chronic Hypoxia and Hypercapnia upon Blood Gases and Acid Base Status[1] (36518)

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There have been numerous studies in which respiration, blood buffering, blood gas tensions, and the like, have been studied during exposure of humans and animals to acute or chronic hypoxia or hypercapnia (1, 2). The combined effect of acute hypoxia–hypercapnia upon respiration and blood gas tensions was studied quite extensively by Gray (3). However, there has been no work to date in which similar studies were made in animals chronically exposed to such an environment. A study of this type is of interest since chronic asphyxia, which can occur in the emphysema patient, for example, is more generally encountered than either hypercapnia or hypoxia alone. A failure of the gaseous control systems in a closed environment, such as an underground shelter or a spacecraft, could also result in both high CO_2 and low oxygen levels.

The present study was designed to measure arterial blood Po_2, Pco_2, pH and standard bicarbonate in rats chronically exposed to a $P_{I_{O_2}}$ of either 70 or 140 Torr in combination with a $P_{I_{CO_2}}$ of 0, 30, 60, or 90 Torr.

Methods. Young adult male Charles River Wistar strain rats were used. They were housed in clear plastic cages, 2 to 3 animals to a cage. Water and Purina laboratory chow were provided *ad libitum*. Pine shavings were used for bedding. The experiments were run in an altitude chamber having an interior volume of about 300 ft^3. It was equipped with a man lock, to facilitate entry with minimal disturbance to the environments. A small instrument lock was available for removal of animals and samples. Total barometric pressure, temperature, relative humidity, and partial pressures of O_2 and CO_2 were automatically controlled and continuously monitored.

For efficient environmental control, total pressure in the chamber was maintained at 380 Torr, relative humidity at 40–60%, and temperature at 24°. The automatic control system generally maintained partial pressures of O_2 and CO_2 to within 2 Torr of the desired setting.

To insure that changes in buffering capacity were complete, the animals were exposed to the experimental environments for 4 days. Previous work has shown that there is little change in standard bicarbonate levels of rats after a 2-day exposure to increased CO_2 levels (4). The rats were exposed in groups of 10 each to the 8 experimental environments for blood gas measurements. A different group of 10 each were exposed for standard bicarbonate determinations. The runs were repeated for some of the environments.

On the fourth day of exposure the rats were anesthetized lightly with an intraperitoneal injection of pentobarbital (50 mg/kg). As soon as they were sufficiently relaxed, an abdominal incision was made and a 20-gauge needle connected to a heparinized glass syringe was inserted into the abdominal aorta for anaerobic blood sampling. The entire time from making the abdominal in-

[1] The research reported in this paper was conducted by personnel of the Environmental Sciences Division, 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.

cision to collecting the blood generally was less than 30 sec. Entry through the lock and the breathing of oxygen through a face mask disturbed the gaseous environment slightly during work in the chamber. While samples were being collected for blood gas measurements, therefore, gas from a cylinder having the exact concentration as the desired chamber environment was allowed to flow through a piece of tubing to a small funnel which was held over the rat's face. Following collection, the samples were capped, placed in ice, and passed through the instrument lock for analysis.

Whole blood P_{O_2}, P_{CO_2}, and pH were measured by an Instrumentation Laboratories blood gas analyzer at 37°. Rectal temperatures of rats exposed to these environments did not differ among groups averaging between 37 and 38°, and a temperature correction was not made. Standard bicarbonate was determined by measuring the pH of the blood after equilibration with two P_{CO_2} levels, using a Radiometer tonometer and pH meter according to the technique of Siggaard-Andersen (5). By definition, standard bicarbonate is the plasma bicarbonate level in blood equilibrated to a P_{CO_2} of 40 Torr and saturated with oxygen. In order to correct our samples to their calculated oxygen saturation, the following correction factor was added to the standard bicarbonate values: std$[HCO_3]$ corrected = std$[HCO_3]$ + Hb g % × 0.3 [(100 − % saturation)/100] (6). This takes into account the fact that oxyhemoglobin is a stronger acid than reduced hemoglobin. The actual bicarbonate was also calculated using the P_{CO_2} and pH after equilibration with the two CO_2 mixtures.

Results. Arterial blood P_{O_2} levels were shown in Fig. 1. In the hypoxic rats[2] the average $P_{a_{O_2}}$ increased from 36.7 Torr, with no added CO_2, to 58.7 Torr with 90 Torr $P_{I_{CO_2}}$. The $P_{a_{O_2}}$ in the normoxic rats increased from 71.8 Torr, with no added CO_2, to 101.2 Torr with 90 Torr $P_{I_{CO_2}}$. The change in $P_{a_{O_2}}$ with increasing $P_{I_{CO_2}}$ was significant in both groups ($p < .01$). The only case where there does not appear to be an increase is between 60 and 90 Torr $P_{I_{CO_2}}$ in the normoxic rats. Arte-

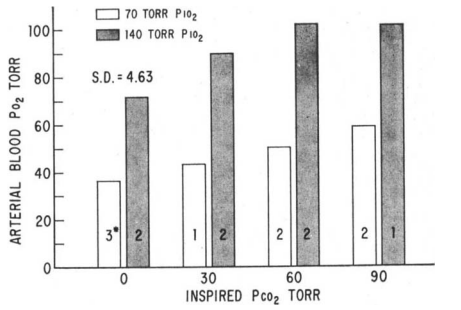

FIG. 1. Arterial blood P_{O_2} of rats exposed 4 days to various levels of inspired O_2 and CO_2. The standard deviation is taken from the mean square error of the analysis of variance on group means. SE can be computed for each environment by dividing by $(n)^{1/2}$ where n = the number of groups of animals included in each mean. *Number of groups of 10 animals each.

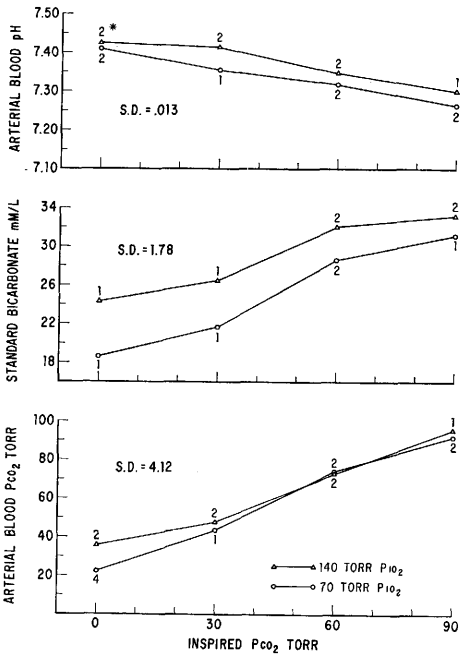

FIG. 2. Arterial blood pH, standard bicarbonate, and P_{CO_2} of rats exposed 4 days to various levels of inspired O_2 and CO_2. SD and SE are computed in the same way as in Fig. 1. *Number of groups of 10 animals each.

[2] Rats inspiring 70 Torr P_{O_2} will be designated as (hypoxic) and those inspiring 140 Torr P_{O_2} as normoxic, recognizing that in the latter case 140 Torr is slightly lower than the $P_{I_{O_2}}$ occurring while breathing air at sea level barometric pressure.

rial blood pH, P_{CO_2}, and standard bicarbonate are shown in Fig. 2. With no inspired CO_2, the arterial blood P_{CO_2} of the hypoxic rats averaged 22.4 Torr, increasing to 91.5 Torr with a $P_{I_{CO_2}}$ of 90 Torr. Normoxic rats averaged 35.8 Torr with a $P_{I_{CO_2}}$ of 0, increasing to 95.3 Torr with a $P_{I_{CO_2}}$ of 90 Torr. Although testing showed a difference between the hypoxic and normoxic rats with no added CO_2 ($p < .01$), general tests using all levels of $P_{I_{CO_2}}$ revealed no significant differences. Standard bicarbonate also increased with increasing $P_{I_{CO_2}}$, but was lower at each level of $P_{I_{CO_2}}$ in the hypoxic rats. This difference, averaged over all levels of $P_{I_{CO_2}}$, was also significant ($p < .05$). As expected, arterial blood pH decreased steadily with increasing $P_{I_{CO_2}}$. No difference was detected between the hypoxic and normoxic rats inspiring no CO_2. With added CO_2 however, the pH of the hypoxic rats dropped below that of the normoxic animals. This difference, averaged over all levels of inspired CO_2, was highly significant ($p < .01$).

A plot of plasma $[HCO_3^-]$ versus pH according to the method of Davenport (7) is shown in Fig. 3. Comparisons are shown between hypoxic and nonhypoxic rats. In all cases the buffer line for the hypoxic rats was displaced downward in comparison with the ones inspiring a normal oxygen level. The slopes of the buffer lines were also increased during inspiration of increased CO_2 levels.

Discussion. Arterial blood P_{O_2} levels were relatively low in the normoxic–nonhypercapnic rats, resulting in a calculated arterial blood oxygen saturation of 94%.[3] This is somewhat lower than the accepted mean values of 96 and 99% for man and dog (8, 9), but agrees well with that reported for unanesthetized rats bled from a cannula in the aortic arch (10). Thus, it appears that rats normally have a low $P_{a_{O_2}}$ and that our experimental animals did not show any noticeable respiratory depression as a result of anesthesia.

The addition of chronic hypercapnia resulted in fairly large increases in $P_{a_{O_2}}$ levels in both the low- and high-oxygen-inspiring groups. Although the beneficial effects of CO_2 upon oxygenation during acute hypoxia have been reported (3), this is the first information, to our knowledge, concerning the chronic state.

Arterial blood P_{O_2} levels increased up to a $P_{I_{CO_2}}$ of 60 Torr in the normoxic rats. The respiratory minute volume is sufficiently high at this point that any further rise, unless large, would not be expected to increase $P_{a_{O_2}}$ levels greatly. There is also some evidence that the respiratory response to CO_2 may be approaching a plateau near this level. For example, cats reach a maximum minute volume while inspiring 10% CO_2. Above this level respiration becomes so intense that expiration is compromised (11). The rat appears to have a very similar minute volume respiration breathing either 5 or 15% CO_2 although at 40 or 50% CO_2, it was increased further (12). This information is for acute exposures, however, and may differ somewhat during chronic exposure.

At the three levels of inspired CO_2 the increased arterial P_{CO_2} was not influenced by hypoxia to a noticeable degree. Evidently, the increased CO_2 stimulates respiration to such an extent and raises $P_{a_{O_2}}$ levels sufficiently that the hypoxic respiratory drive causes little further increase in minute volume. However, standard bicarbonate and pH levels of the arterial blood were lower in the hypoxic than in the nonhypoxic rats inspiring the same level of CO_2. It has generally been believed that bicarbonate levels merely adjust to changes in $P_{a_{CO_2}}$ during either hypoxia or hypercapnia. During hypercapnia, for example, blood bicarbonate levels increase to buffer the rise in $[H^+]$ due to increased CO_2 levels (13). With hypoxia, on the other hand, $P_{a_{CO_2}}$ declines as a result of greater ventilation and bicarbonate levels subsequently decrease to alleviate potential alkalosis (14–16). This compensation is incomplete, particularly

[3] Saturation was determined with a radiometer blood gas calculator which uses the oxygen saturation curve for men. This could result in a small error in our estimate.

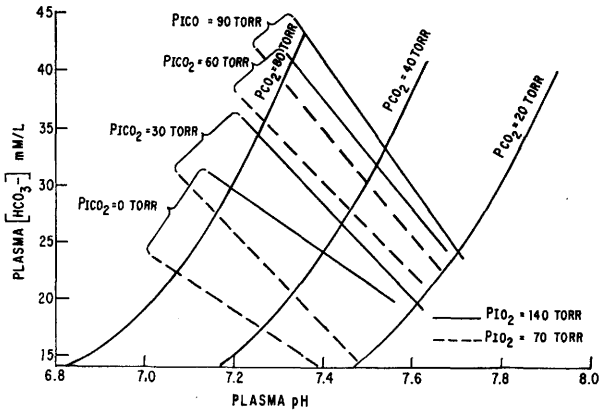

FIG. 3. Comparisons of plasma bicarbonate versus pH. Comparisons are shown between hypoxic and nonhypoxic rats exposed to 0, 30, 60 and 90 Torr $P_{I_{CO_2}}$. In addition, calculated isopleths for 20, 40 and 80 Torr arterial P_{CO_2} are included.

during chronic hypercapnia. In the chronic hypercapnic dog, the following relationship has been reported for arterial blood: $[H^+] \times 10^{-9} = 26.9 + 0.32\ P_{a_{CO_2}}$ (17). Data from the present experiment for normoxic rats yielded the relationship: $[H^+] \times 10^{-9} = 29.3 + 0.22\ P_{a_{CO_2}}$, indicating that rats compensate slightly better than dogs. It is apparent from the present data, however, that hypoxia, in some way, partially prevents the compensatory increase in bicarbonate buffering during hypercapnia. Furthermore, this effect is not the result of lower arterial blood P_{CO_2} levels in the hypoxic animals.

The slopes of the buffer lines in the pH – bicarbonate diagram are greater than normal for all groups inspiring increased CO_2 and appear to be even steeper at the highest CO_2 level. The slope is a measure of nonbicarbonate buffers in the blood, mainly hemoglobin and plasma proteins (7). All the environments used with the exception of the controls result in hyperventilation and subject the animals to various degrees of stress. This can result in severe dehydration and release of additional red blood cells into the circulation, increasing the concentration of hemoglobin and plasma proteins (18).

The displacement of the buffer lines represents a difference in the bicarbonate buffer levels in the blood at a given P_{CO_2}. Addition of an acid or base other than carbonic acid will shift these buffer lines downward or upward, respectively, without changing the slope. The data shown on the pH – bicarbonate diagram was not corrected for differences in oxygen–hemoglobin saturation. Such a correction would result in the buffer lines of the hypoxic rats shifting downward even further since oxygenated hemoglobin is a stronger acid than reduced hemoglobin.

A downward displacement of the buffer lines may have been due to increased production of acid by the animal. For example, some of these environments may be sufficiently stressful for the animals to stop eating, resulting in ketosis. Lactic acid may also increase. Although plasma lactic acid shows only a transient increase during hypoxia (19) there are no data during chronic hypoxia–hypercapnia. Eight blood samples from rats exposed 4 days to each environment were assayed for lactic acid and ketones. The lactic acid values were quite variable, a finding not uncommon in the rat (20) and no conclusions could be drawn. The hypoxic rats did have significantly higher ketone levels at 30 and 60 Torr $P_{I_{CO_2}}$ than the normoxic ones, but the differences were small and probably not important.

Another factor which may affect bicarbonate buffering is kidney function. For example, it is well known that carbonic anhydrase inhibition or increases in plasma Cl, K, or adrenal cortical hormones will depress bicarbonate reabsorption (21). At present,

however, there is no information concerning levels of these substances during chronic hypoxia–hypercapnia. Thus, the underlying mechanisms influencing buffering during chronic hypoxia–hypercapnia are not yet completely known.

Summary. Young adult male Charles River rats were exposed for 4 days, in groups of 10 each, to an inspired P_{CO_2} of 0, 30, 60 and 90 Torr, combined with an inspired P_{O_2} of 70 or 140 Torr. Arterial P_{O_2} in the rats inspiring a P_{O_2} of 70 Torr increased from a mean of 37 Torr, with no added CO_2, to 59 Torr with a $P_{I_{O_2}}$ of 90 Torr. In the rats breathing normal oxygen levels (140 Torr), arterial blood P_{O_2} increased from 72 Torr, with no added CO_2, to 101 Torr with a $P_{I_{CO_2}}$ of 90 Torr. Standard bicarbonate increased with increasing $P_{I_{CO_2}}$ in all groups; however, it was consistently lower in the low-oxygen-breathing rats than in the normoxic animals ($p < .05$). On the other hand, arterial P_{CO_2} was significantly lower in the hypoxic rats, owing to hypoxic hyperventilation, only when inspired CO_2 levels were 0. This resulted in a lower arterial blood pH in the hypoxic than in the nonhypoxic rats inspiring comparable CO_2 levels ($p < .01$).

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