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Effect of prolonged exposure to 0.5% CO_2 on kidney calcification and ultrastructure of lungs

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Schaefer, K. E., W. H. J. Douglas, A. A. Messier, M. L. Shea, and P. A. Gohman. 1979. Effect of prolonged exposure to 0.5% CO_2 on kidney calcification and ultrastructure of lungs. Undersea Biomed. Res. Sub. Suppl.: S155–S161.—Guinea pigs were exposed for up to 8 weeks to 0.5% CO_2, 21% O_2, and balance N_2. Control groups of the same age were kept simultaneously in environmental chambers on air. A slight increase in Pa_{CO_2} and decrease in pH were observed at various periods of exposure to 0.5% CO_2. After eight weeks of exposure, an increased kidney calcification, indicated by increased kidney calcium content, was found. Plasma calcium was significantly elevated at this point, apparently because of the release of calcium from bone. After 8 weeks of recovery on air that followed 8 weeks of exposure to 0.5% CO_2, values had returned to control levels. No significant ultrastructural changes were found in the lungs after 4, 6, and 8 weeks of exposure to 0.5% CO_2.

CO_2	metabolism
kidney function	bones
calcium	lungs

Studies of guinea pigs exposed for prolonged periods to 1% CO_2 showed two important findings: 1) increased kidney calcification, as measured in kidney calcium content (Schaefer, Pasquale, Messier, and Niemoeller 1979) and 2) ultrastructural changes of the lungs involving a proliferation of pneumocyte II cells, considered to be precursors of alveolar lining cells (Type I cells) and the site of surfactant production (Douglas, Schaefer, Messier, and Pasquale 1979).

Findings of increased kidney calcium content during exposure to 1% CO_2 corroborated previous histopathological studies of guinea pigs which demonstrated increased focal calcification in the kidney after exposure to 1.5% CO_2 (Schaefer et al. 1979).

The observed changes in lung structure have been interpreted as compensatory reactions to the impairment caused by CO_2 on the alveolar lining cells (Douglas et al. 1979).

Studies of the effects of prolonged exposure to 1% CO_2 demonstrated that the observed changes in lungs and kidneys can provide criteria for establishing threshold effects of chronic CO_2 exposure on these target organs in animals. To determine threshold effects of chronic low level hypercapnia, further studies were carried out in which guinea pigs were exposed for periods of up to 8 weeks to 0.5% CO_2, 21% O_2, and balance N_2.

MATERIALS AND METHODS

Two experiments were carried out in which guinea pigs were exposed for 8 weeks to 0.5% CO_2, 21% O_2, and balance N_2. In the first experiment, ultrastructural changes of the lungs were observed after 8 weeks of exposure in one group of animals only. In view of the importance and possible implications of this finding, it was deemed necessary to confirm the results and to repeat the experiment. Moreover, corresponding blood and tissue calcium data had not been obtained for the 8 weeks of exposure in the first experiment.

In both experiments, caesarean-section-born male guinea pigs of the Hartley strain were used. They had been maintained free of respiratory disease and weighed between 400 and 500 g at the start of the experiment. They were exposed to 0.5% CO_2, 21% O_2, and balance N_2 in environmental chambers in which the temperature was kept at 25.0 ± 1.1°C and the humidity between 65 and 75%. The gas mixtures were prepared by mixing proportional amounts of CO_2 to air; oxygen was added from a high pressure cylinder. With this quick and large turnover of chamber air, mixing of CO_2 and air was nearly instantaneous. The carbon dioxide concentration in the chamber was continuously monitored with a Beckman infrared CO_2 analyzer.

A complete gas analysis (CO_2, O_2, N_2) was carried out on a daily basis with a Hewlett-Packard 7620A gas chromatograph. The CO_2 concentrations were kept at 0.5% ± 0.1% and the oxygen concentration at 21% ± 0.5%. Ammonia vapor was absorbed by boric acid in the chamber. The chamber was opened each morning for a period of 3–5 min to fill the water and food containers and to remove urine and feces.

Littermates of the animals exposed to CO_2 were kept in a second environmental chamber under identical conditions except that the ambient air was free of CO_2. For each experimental period, six animals exposed to CO_2 were killed, together with four control animals.

Prior to blood sampling, the animals received 40 mg pentobarbital/kg body wt intraperitoneally and were returned to the CO_2 exposure chamber. The anesthesia was usually effective after approximately 5 min, at which time the animals were taken out of the exposure chamber and immediately placed under a mask through which they breathed the same CO_2 gas mixtures to which they had been exposed. Blood samples were drawn from the abdominal aorta. Blood pH and P_{CO_2} were determined with an Instrumentation Laboratory blood gas and pH analyzing system. The femurs of both legs were removed, rapidly cleaned, and stripped free of adhering tissue and bone marrow. Specimens of compact bone between 200–300 mg were kept on ice for determination of bone electrolytes. The time between procurement and analysis of the fresh samples did not exceed two hours. Paired specimens were oven-dried to a constant weight at 150°C for 18 h before analysis.

Body temperature was measured just prior to blood sampling and pH and P_{CO_2} values were corrected to body temperature. Plasma, bone, and kidney calcium were measured with an atomic absorption spectrometer. For calcium determination, kidney tissues were dry-ashed at 650°C for 24 h; bone samples were dissolved in 6N nitric acid at 65°C. Appropriate dilutions of the digest made with lanthanum oxide were used for analysis. Recovery was assessed by adding known amounts of calcium to standard solutions.

Electron microscopic studies of lung ultrastructure

Blocks of lung tissue from control and exposed animals were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Details of the fixation method have been described previously (Redding, Arai, Douglas, Tsurutani, and Oven 1975). Tissue specimens were evaluated for ultra-

structural changes of the lung, i.e., alterations of cell membranes, number, size, and distribution of intracellular structures.

All statistical comparisons of physiological and morphological data were done by Student's *t*-test.

RESULTS

Results are presented of the second experiment, in which complete data were collected. However, in the discussion, reference is made to data obtained in the first experiment. Figure 1 exhibits data on body weight, P_{CO_2}, pH, and standard bicarbonate in animals exposed to 0.5% CO_2 and in control animals. Blood samples included in the table are those in which the P_{O_2} values were above 50 mmHg. The weights of experimental and control animals corresponded well with each other. There was a slight elevation of P_{CO_2} of about 2–3 mmHg in exposed animals compared to control animals. The pH was slightly lower in the exposed animals. No consistent change was found in standard bicarbonate levels.

Data on bone calcium, plasma calcium, and kidney calcium are displayed in Fig. 2. Bone calcium tended to decrease after 6 and 8 weeks of exposure. At the lowest bone calcium level,

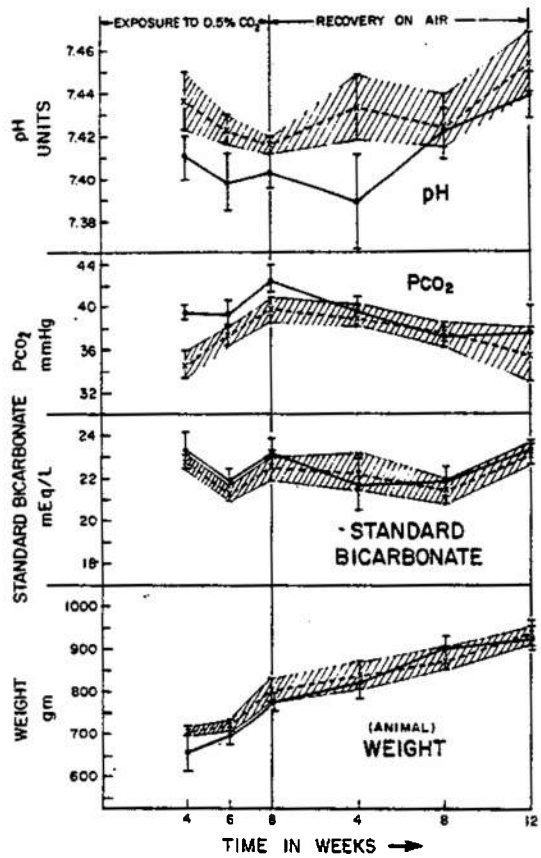

Fig. 1. Effect of prolonged exposure to 0.5% ± 0.1% CO_2, 21% ± 0.5% O_2, and balance N_2 on pH, P_{CO_2} (mmHg), standard bicarbonate (mEq/liter) of arterial blood, and body weight of guinea pigs; •——• = exposed to 0.5% CO_2; X----X = controls in environmental chamber on air. Cross-hatched area shows SEM of controls; ⊙ = significantly different from controls killed at the same time.

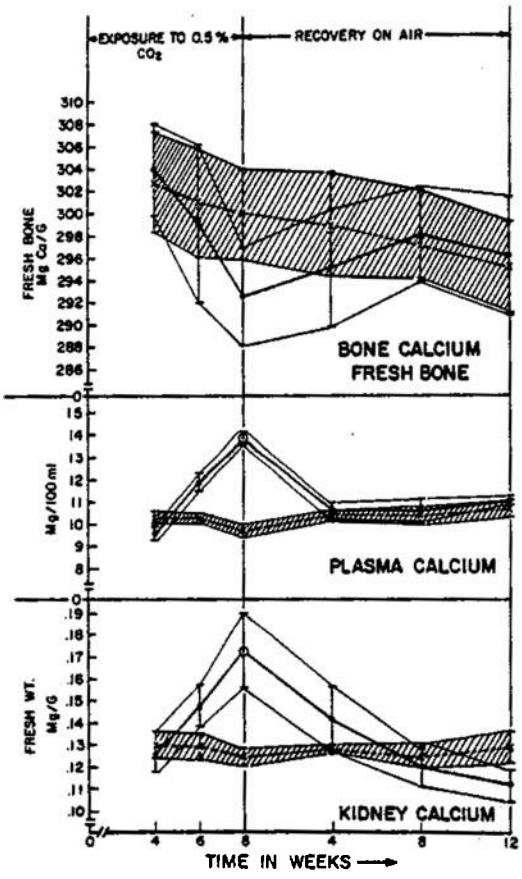

Fig. 2. Effect of prolonged exposure to 0.5% ± 0.1% CO_2, 21% ± 0.5% O_2, and balance N_2 on bone calcium concentrations (mg/g wet bone) plasma calcium (mg/100 ml) and kidney calcium content (mg/g wet tissue); •——• = exposed to 0.5% CO_2; X----X = controls in environmental chamber on air. Cross-hatched area shows SEM of controls; ⊙ = significantly different from controls killed at the same time.

at 8 weeks of exposure, a marked increase in plasma calcium occurred, which was paralleled by a statistically significant rise in kidney calcium content. During the recovery period on air after 8 weeks of exposure to 0.5% CO_2, all values returned to control levels.

Data on cell diameter, number of lamellar bodies, and lamellar body diameters of Type II pneumocytes in guinea pigs exposed to 0.5% CO_2 and controls obtained in the experiment are given in Table I. No significant changes were observed. Plasma K, Na, and Cl concentrations determined in both experiments remained at control levels. No significant changes were observed in body temperature.

DISCUSSION

Before evaluating the results of the experiments, it is necessary to assess the influence of the anesthetic on the acid-base status of the animals. Pontén and Siesjo (1967) measured pH in rats prior to injection of barbiturate and after attainment of surgical anesthesia and found a decrease of 0.07 pH units. Since the latter conditions were imposed on both control and experi-

TABLE 1

EFFECTS OF PROLONGED EXPOSURE TO 0.5% CO_2 ON ULTRASTRUCTURE OF THE LUNGS

Time	Exposed to 0.5% CO_2			Controls		
	Cell Diameter, mm	Lamellar Bodies per Cell	Lamellar Bodies, Diameter, mm	Cell Diameter, mm	Lamellar Bodies per Cell	Lamellar Bodies, Diameter, mm
4 Weeks	9.0 (0.2)	5.2 (0.08)	1.2 (0.09)	9.2 (0.09)	4.9 (0.07)	1.1 (0.08)
6 Weeks	9.1 (0.3)	5.1 (0.15)	0.9 (0.08)	8.9 (0.5)	5.2 (0.13)	1.0 (0.07)
8 Weeks	9.1 (0.9)	5.1 (0.3)	1.0 (0.08)	9.2 (0.6)	5.0 (0.2)	0.9 (0.06)
8 Weeks' Exposure + 8 Weeks' Recovery	9.1 (0.8)	4.8 (0.08)	1.0 (0.01)	9.0 (0.2)	5.2 (0.1)	1.3 (0.04)
8 Weeks' Exposure + 12 Weeks' Recovery	8.8 (0.6)	5.1 (0.3)	1.0 (0.08)	8.9 (0.9)	4.9 (0.7)	1.1 (0.1)

Experimental group, $n = 6$; control, $n = 4$.

mental animals, all the measured blood pH values observed in our experiments are a little too low, but the time course of pH changes observed during prolonged exposure to low levels of CO_2 would not be altered by the anesthesia effects.

The differences in Pa_{CO_2} and pH between exposed and control animals were so small that the accuracy limits of blood gas and pH measurements were approached. Nevertheless, there was enough evidence indicating that exposure to 0.5% CO_2 caused a slight rise in blood CO_2 tension and pH. In both experiments, no significant elevations of standard bicarbonate were found, indicating that the kidney does not increase renal bicarbonate reabsorption in response to this small elevation of Pco_2 and that a metabolic acidosis occurs similar to but less pronounced than that observed during exposure to 1% CO_2. No changes in plasma K were observed in either experiment with 0.5% CO_2. These results suggest that alterations in K metabolism did not contribute to the acidosis.

Kidney calcification occurred during exposure to 0.5% CO_2 after 8 weeks, which represents a marked delay in onset compared to the effect of 1% CO_2, where kidney calcification was observed after two weeks of elposure.

The decline of bone calcium during the latter part of the exposure to 0.5% CO_2 (6 and 8 weeks) and the corresponding rise in plasma calcium are similar to the findings observed during the same periods of exposure to 1% CO_2. It seems, therefore, that 0.5% CO_2 still exerts an effect on bone-blood calcium exchange that results in kidney calcification.

However, in contrast to the altered ultrastructure of the lungs found after four weeks of exposure to 1% CO_2 and after four weeks of recovery on air after exposure (Douglas et al. 1979), no changes in ultrastructure were observed at 4, 6, and 8 weeks of exposure to 0.5% CO_2 in the second experiment. The occurrence of such ultrastructural changes in a single group of animals after 8 weeks of exposure to 0.5% CO_2 in the first experiment must have been caused by factors other than CO_2.

The guinea pigs used in this study were much more sensitive to CO_2 than rats, which is reflected in physiological and histopathological effects (Schaefer, Niemoeller, Messier, Heyder, and Spencer 1971). In view of the existing species differences, it would seem important to determine whether rats show any lung ultrastructure effects after prolonged exposure to 0.5% CO_2.

Schaefer, K. E., W. H. J. Douglas, A. A. Messier, M. L. Shea, and P. A. Gohman. 1979. Effets d'une exposition prolongée à 0,5% CO_2 sur la calcification rénale et sur l'ultrastructure pulmonaire. Undersea Biomed. Res. Sub. Suppl.: S155–S161.—On a exposé des cobayes à un mélange de 0,5% CO_2, 21% O_2, reste N_2. Des groupes d'animaux témoins ont passé les mêmes périodes en caissons environmentaux à l'air. On a observé une augmentation légère de Pa_{CO_2} et une diminution de pH à certains moments au cours de l'exposition. Après huit semaines d'exposition, on a observé une calcification rénale augmentée, c'est-à-dire une concentration augmentée de calcium au niveau des reins. A ce moment, la calcémie est aussi augmentée, sans doute à cause de la libération de calcium des os. Après huit semaines de rétablissement en air, qui ont suivi les huit semaines d'exposition, les concentrations sont revenues à leurs valeurs normales. Aucune modification significative de l'ultrastructure pulmonaire n'été observée après quatre, six, ou huit semaines d'exposition.

CO_2
fonction rénale
calcium
métabolisme
os
poumons

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