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# Steady-state gas exchange in normothermic, anesthetized, liquid-ventilated dogs

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Matthews, W. H., R. H. Balzer, J. D. Shelburne, P. C. Pratt, and J. A. Kylstra. 1978. Steady-state gas exchange in normothermic, anesthetized, liquid-ventilated dogs. Undersea Biomed. Res. 5(4): 341–354.—Anesthetized, paralyzed, purebred beagle dogs were ventilated for 45 min ($n=5$) and 60 min ($n=5$) with oxygenated ($P_{I_{O_2}}$ = 685 mmHg) FC-80 fluorocarbon liquid at 38°C. The $Pa_{CO_2}$ remained constant at approximately 43 mmHg during 60 min of liquid ventilation (mean tidal volume = 290 ml, mean respiratory frequency = 2.8 breaths/min). The end-tidal $P_{CO_2}$ was consistently higher than $Pa_{CO_2}$ during liquid ventilation. Histological examination by light as well as scanning electron microscopy of the lungs of dogs killed 10, 30, or 180 days after liquid ventilation revealed no pathological changes except for a slight increase in the number of macrophages, especially around the alveolar ducts. It is concluded that a steady state of gas exchange at near normal arterial carbon dioxide partial pressures can be maintained in anesthetized, normothermic dogs ventilated with FC-80 at respiratory frequencies of approximately 3 breaths/min.

liquid breathing
negative $P(a\text{-}A)_{CO_2}$
gas exchange

The effects of pressure on marine organisms, isolated organs, and cells have been studied since 1884 (Johnson, Eyring, and Polissar 1954). The response to uniform compression of gas-breathing intact animals and man may be affected by pharmacological properties of compressed gases or physico-chemical sequelae of transient inert gas pressure gradients (Kylstra, Longmuir, and Grace 1968; Graves, Idicula, Lambertsen, and Quinn 1973). Hydraulic compression of liquid-breathing mice has been shown to elicit tremors, uncoordinated limb movements, tonic convulsions (Kylstra, Nantz, Crowe, Wagner and Saltzman 1967), as well as a decrease in heart rate and respiratory frequency (Lundgren and Örnhagen 1976), but these animals were not maintained at normal body temperature, and pressure-induced biological phenomena are temperature-dependent (Fenn 1967). Moreover, liquid-breathing mammals usually develop a respiratory acidosis that might also modify responses to hydrostatic compression. To determine the effects of pressure per se, potential artifacts must be avoided. Hence, one would like to be able hydraulically to compress liquid-breathing mammals with

normal body temperatures, normal arterial blood gases, and normal acid-base balances. We report here on experiments in which it was possible to maintain a near normal $Pa_{CO_2}$ in anesthetized normothermic beagle dogs that were ventilated for 60 min with oxygenated perfluoro-butyltetrahydrofuran, FC-80 fluorocarbon liquid (3M Co., St. Paul, Minn.).

## METHODS

Purebred male beagle dogs that had not eaten for 24 h but had received water ad libitum were anesthetized with sodium pentobarbital (30 mg/kg) intravenously and were ventilated mechanically with bubble-oxygenated FC-80 fluorocarbon liquid. Five dogs (*group A;* weight = 10.8 ± 1.1 kg; mean ± SD) were ventilated for 60 min at a respiratory frequency of 2.8 ± 0.3 breaths/min, a tidal volume of 290 ± 3 ml, and an FRC of 479 ± 26 ml. To assess the effect of respiratory frequency on diffusive mixing in the alveoli (see DISCUSSION), five other dogs (*group B;* weight = 11.0 ± 2.6 kg) were ventilated for 45 min at a respiratory frequency of 5.5 ± 0.4 breaths/min, a tidal volume of 181 ± 8 ml, and an FRC of 461 ± 25 ml. The tidal volume in *group B* was less than that in *group A*, to obtain an approximately equal alveolar minute ventilation in both groups of dogs.

The apparatus used for liquid ventilation is shown in Fig. 1. The FC-80 fluorocarbon liquid was kept in two air-tight reservoirs; one reservoir (A) was a 56.8-liter Nalgene synthetic polymer container placed in a water bath (B). The other reservoir (C) was an 18.9-liter Nalgene box which was placed above the dog. Fluorocarbon liquid was pumped continuously from reservoir (A) to reservoir (C) by a centrifugal pump (D). The vertical distance between the level of the fluorocarbon liquid in reservoir (C) and the dog remained constant as a result of overflow (F). The temperature of the water bath was regulated so that the temperature of the fluorocarbon in the upper reservoir was maintained at 38.0 ± 0.5°C, as measured by a tele-

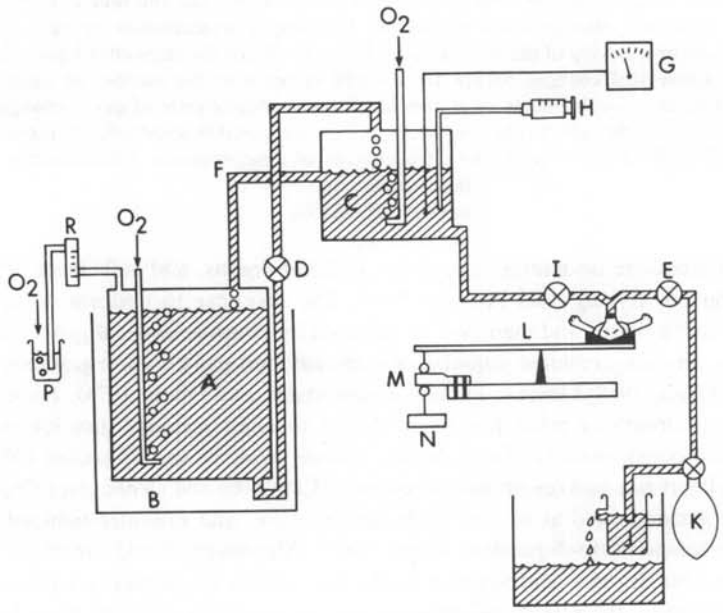
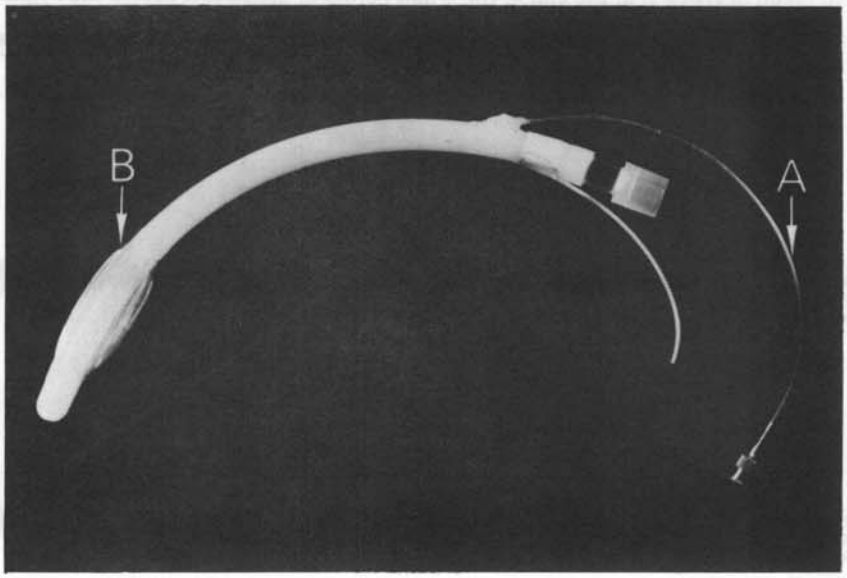



Fig. 1. Schematic diagram of experimental apparatus used in liquid ventilation experiments.

thermometer (G). Oxygen was bubbled through the FC-80 in each reservoir for at least 12 h prior to each experiment at a rate of 1–2 liters/min as measured by a flowmeter (R) connected to a water seal (P). The oxygen flow was discontinued one-half hour before liquid ventilation began. The absence of any gas bubbles in the inspired FC-80 was ascertained by close inspection just before the experiment. The inspired partial pressures of $O_2$ and $CO_2$ ($P_{I_{O_2}} = 685 \pm 13$ mmHg; $P_{I_{CO_2}} = 4.6 \pm 0.6$ mmHg) were measured in samples of FC-80 fluorocarbon taken through tube (H). The $P_{I_{CO_2}}$ was as high as 4.6 mmHg because the fluorocarbon obtained commercially contained surprisingly large amounts of dissolved $CO_2$ which could not all be removed with the oxygen-bubbling technique used in the experiments. The flow of FC-80 into and out of the lungs of the dog was controlled by two solenoid-operated clamps (I and E). The fluorocarbon was drained from the lungs by gravity into a small beaker (J) from which it overflowed into a larger reservoir. Thus, the height of the fluorocarbon column in the expiratory line remained constant with respect to the dog. A 3-liter rubber bag (K) was connected to the expiratory line through a valve so that mixed expired fluorocarbon samples could be collected.

The dog was placed on a metal tray which was attached to the sample platform of a balance (L). The tip of the arm of the balance was connected by thin wire to a force displacement transducer (M) that was attached to the base of the balance. The transducer output was amplified with a low-level DC preamplifier and recorded on a polygraph recorder. Weights (N) were attached to the transducer to counterbalance the weight of the dog so that the output of the transducer was zero while the dog was ventilated with room air. Before filling the dog's lungs with liquid, the deflection of the recording pen was calibrated by placing standard weights on the tray and converting mm of pen deflection into volumes of FC-80 by taking into account the density of FC-80 at 38°C (1.725 g/ml; Technical information, 3M Co.).

After the anesthetic was administered, a 9.0-mm cuffed endotracheal tube (Fig. 2) was placed in the dog's trachea. A small-bore catheter (A) was placed in the lumen of the endo-



tracheal tube, and its tip advanced distally to the position shown by the arrow (B). The location of the tip of the catheter in the distal trachea permitted representative alveolar gas and liquid samples to be collected. Rectal temperature was monitored continuously from the onset of anesthesia to the completion of liquid ventilation with a rectal probe connected to a telethermometer. The temperature of the dog was maintained at 38.1 ± 0.3°C with the aid of a heating pad placed under the animal. Using sterile technique, the right femoral vessels were exposed. A No. 18 2-in. Teflon catheter was placed in the right femoral artery, without ligating the artery, so that blood flow would be uninterrupted. A No. 4 French Swan-Ganz catheter was placed in the femoral vein and its tip advanced into the pulmonary artery, as indicated by the pressure tracings. Femoral and pulmonary arterial pressures were sensed by pressure transducers and recorded continuously.

The dog was paralyzed with pancuronium bromide administered intravenously (0.08 mg/kg), and ventilated mechanically with room air for at least 15 min. Arterial and mixed venous blood, end-tidal and mixed expired gas samples were taken. The dog was then ventilated with 100% oxygen for at least 15 min at the same tidal volume and frequency.

The gas respirator was subsequently disconnected and the endotracheal tube connected to the liquid ventilation apparatus. FC-80 fluorocarbon was introduced into the lungs of the dog at a rate of 100 ml/min until a volume equal to the estimated FRC (Mauderly 1974) was reached, as indicated by the deflection of the pen of the recorder. The deflection of the pen caused by priming the lungs with fluorocarbon was then reset to the base-line position on the chart. This allowed the use of full-scale pen deflection for the recording of tidal volumes, which increased the sensitivity of the recording. A photograph of a tracing from an experiment is shown in Fig. 3.

Arterial and mixed venous blood, and inspired, mixed expired, and end-tidal FC-80 samples were taken 10, 20, 30, 45, and 60 min after the beginning of liquid ventilation in *group A* and 10, 20, 30, and 45 min after the beginning of liquid ventilation in *group B*. Arterial blood samples were also taken in *group B* at the end of the 4-min period of apnea just before the beginning of

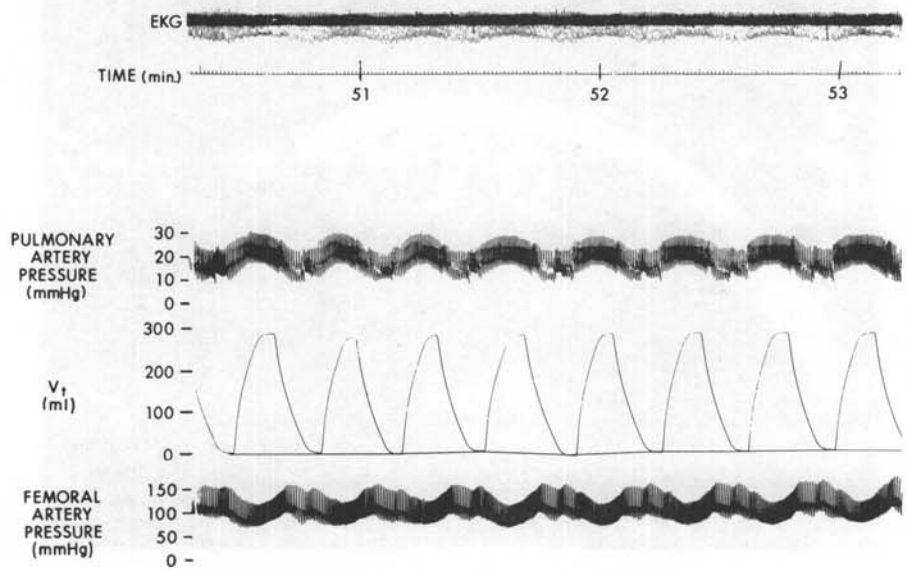



Fig. 3. Photograph of representative chart from liquid ventilation experiment on beagle from *group A* (f = 2.8 breaths/min).

liquid ventilation.

After the completion of liquid ventilation, muscle paralysis was abolished by the intravenous administration of 1.0 mg neostigmine and 0.5 mg atropine. The expiratory reserve volume of liquid was drained from the dog's lungs by gravity. All catheters were removed from the femoral vessels and the wound was closed. No antibiotics were given. The dog was ventilated manually with 100% $O_2$ until spontaneous respirations were judged to be adequate, placed in a closed cage, and made to breathe 100% and 60% $O_2$ for 8 and 12 h, respectively, before being allowed to breathe room air again.

The FC-80 fluorocarbon liquid was washed with water and filtered after each experiment and then used again for the next experiment.

Partial pressures of oxygen and carbon dioxide in gas, blood, and FC-80 fluorocarbon samples were measured with Clark and Severinghaus electrodes. The pH of the blood samples was measured with a Radiometer electrode. All electrodes were immersed in a water bath maintained at 37°C. All blood gas and pH measurements were corrected to the body temperature of the dog with a Radiometer BGCl Blood Gas Calculator with extended cursor.

The oxygen and carbon dioxide electrodes were calibrated for measurement of $P_{O_2}$ and $P_{CO_2}$ in FC-80 fluorocarbon in the following manner: 50-ml samples of FC-80 were placed in a tonometer in a water bath which was maintained at 37°C. A calibrating gas mixture containing 97% $O_2$ and 3% $CO_2$ was bubbled through the FC-80 for 7 h. The calibrating gas mixture (analyzed by the Scholander micro gas-analysis technique) was introduced into the oxygen electrode and the pointer of the Instrumentation Laboratory 113 Electrometer set to the computed $P_{O_2}$ of the calibrating gas mixture. A 4-ml sample of FC-80 was then drawn anaerobically from the tonometer and introduced into the oxygen electrode. The computed $P_{O_2}$ of the calibrating gas mixture divided by the recorded $P_{O_2}$ of the FC-80 sample was taken to be the $O_2$ calibration factor. Nine such measurements were made on three days and the mean $\pm$ SD $O_2$ calibration factor was $1.0974 \pm 0.0116$. For determination of the $CO_2$ calibration factor, a gas mixture containing 5% $CO_2$, 70% $O_2$, and 20% $N_2$ was bubbled through 50-ml samples of FC-80 for 7 h and the same procedure as described above was followed. The mean $CO_2$ calibration factor obtained from 11 measurements made on three days was $1.0406 \pm 0.0067$. All $P_{O_2}$ and $P_{CO_2}$ measurements of FC-80 samples taken during the experiment were multiplied by the respective correction factors.

Three other beagle dogs (*group C)* were anesthetized, paralyzed, and ventilated mechanically with room air for approximately 3 h after the onset of anesthesia. All other conditions and procedures were the same as described above.

To determine the statistical significance of differences between data obtained in one group of dogs, the paired-$t$ test was used. The $t$ test for two means was used to determine the statistical significance of the differences between measurements obtained in two different groups of dogs. Differences in means from these data resulting in a $P \leq 0.05$ were judged to be significant.

Dogs were killed 1, 10, 30, or 180 days after breathing FC-80 fluorocarbon. Two control dogs, one purebred beagle and one mongrel, were ventilated with the Harvard respirator for approximately 2 h and then placed in a closed cage and made to breathe 100% and 60% $O_2$ for 8 and 12 h, respectively, before being allowed to breathe room air. These two dogs were then killed.

The lungs were removed and prepared for examination with both light and scanning electron microscope. For light microscope examination, portions of each lung were fixed by immersion in 10% neutral buffered formalin, followed by paraffin embedding and hematoxylin and eosin staining of 6-$\mu$m sections. For examination with the scanning electron microscope, the right

upper lobe of each lung was fixed by perfusion via the bronchus with 0.1 M cacodylate-buffered 3% glutaraldehyde. After fixation for various lengths of time, aliquots of this tissue were critically point-dried, using a graded series of ethanol and Freon 13. Sections were placed on pure carbon stubs, and were evaporation-coated with platinum carbon before scanning electron microscopic examination (Ingram, Morosoff, Pope, Allen, and Tisher 1976).

## RESULTS

The results obtained in *group A* and *group B* are given in Tables 1 and 2. The mean $Pa_{O_2}$ in *group A* ranged from 295 to 316 mmHg during 60 min of liquid ventilation. In *group B*, the mean $Pa_{O_2}$ ranged from 204 to 221 mmHg during 45 min of liquid ventilation. The differences between the mean $Pa_{O_2}$ in *group A* and *group B* were statistically significant ($P \leq 0.02$) except

**TABLE 1**
DATA FROM GROUP A DOGS

| | | Ventilation with FC-80 Fluorocarbon, Elapsed Time, min | | | | |
|---|---|---|---|---|---|---|
| | Air | 10 | 20 | 30 | 45 | 60 |
| f, breaths/min | 12.4 ±0.7 | 2.8 ±0.4 | 2.7 ±0.4 | 2.8 ±0.3 | 2.8 ±0.3 | 2.8 ±0.3 |
| $\dot{V}_E$ ml(BTPS)/min | 3404 ±159 | 811 ±79 | 787 ±118 | 812 ±91 | 803 ±93 | 804 ±87 |
| $\dot{V}_A$ ml(BTPS)/min | 2262 ±271 | 589 ±92 | 580 ±91 | 523 ±84 | 548 ±74 | 569 ±59 |
| $Pa_{O_2}$, mmHg | 98 ±6 | 316 ±29 | 298 ±22 | 295 ±29 | 315 ±28 | 303 ±21 |
| $Pa_{CO_2}$, mmHg | 29.7 ±4.2 | 42.6 ±4.4 | 44.8 ±3.5 | 42.4 ±3.9 | 40.5 ±3.3 | 44.2 ±3.7 |
| $pH_a$ | 7.42 ±.06 | 7.28 ±.03 | 7.27 ±.03 | 7.27 ±.07 | 7.30 ±.03 | 7.27 ±.05 |
| Base Excess, mEq/liter | −5 ±3 | −7 ±3 | −6 ±3 | −7 ±5 | −6 ±2 | −7 ±2 |
| $PET_{O_2}$, mmHg | 114 ±9 | 463 ±28 | 452 ±28 | 448 ±24 | 445 ±28 | 450 ±58 |
| $PET_{CO_2}$, mmHg | 27.5 ±5.5 | 43.4 ±4.3 | 46.0 ±3.4 | 48.2 ±2.8 | 46.9 ±5.2 | 47.8 ±4.1 |
| $PE_{O_2}$, mmHg | 125 ±4 | 532 ±36 | 569 ±44 | 557 ±51 | 566 ±42 | 573 ±41 |
| $PE_{CO_2}$, mmHg | 18.3 ±1.6 | 30.5 ±3.9 | 32.1 ±1.6 | 31.9 ±4.1 | 31.6 ±4.8 | 32.7 ±2.6 |
| $P(ET\text{-}a)_{O_2}$, mmHg | 16 ±15 | 147 ±24 | 154 ±34 | 154 ±42 | 140 ±51 | 147 ±54 |
| $P(a\text{-}ET)_{CO_2}$, mmHg | 2.4 ±1.8 | −1.1 ±6.2 | −2.1 ±3.8 | −5.8 ±5.3 | −6.4 ±3.2 | −3.6 ±5.3 |

Values are means ± SD; $n = 5$.

**TABLE 2**
DATA FROM GROUP B DOGS

| | | Ventilation with FC-80 Fluorocarbon, Elapsed Time, min | | | |
|---|---|---|---|---|---|
| | Air | 10 | 20 | 30 | 45 |
| f, breaths/min | 11.5 ±0.9 | 5.7 ±0.5 | 5.3 ±0.2 | 5.6 ±0.3 | 5.6 ±0.4 |
| $\dot{V}_E$, ml(BTPS)/min | 3208 ±242 | 1048 ±128 | 951 ±48 | 995 ±88 | 1017 ±81 |
| $\dot{V}_{A_e}$, ml(BTPS)/min | 2007 ±217 | 574 ±203 | 546 ±109 | 519 ±105 | 549 ±104 |
| $Pa_{O_2}$, mmHg | 81 ±6 | 204 ±46 | 206 ±47 | 221 ±72 | 215 ±69 |
| $Pa_{CO_2}$, mmHg | 32.1 ±2.0 | 44.8 ±7.1 | 46.2 ±5.8 | 47.7 ±3.9 | 50.0 ±10.3 |
| $pH_a$ | 7.41 ±.03 | 7.28 ±.05 | 7.24 ±.08 | 7.27 ±.04 | 7.23 ±.12 |
| Base Excess, mEq/liter | −4 ±2 | −6 ±1 | −7 ±5 | −5 ±2 | −7 ±5 |
| $P_{ET_{O_2}}$, mmHg | 114 ±5 | 422 ±44 | 375 ±19 | 406 ±41 | 393 ±23 |
| $P_{ET_{CO_2}}$, mmHg | 28.8 ±2.6 | 45.5 ±3.9 | 49.3 ±5.0 | 52.5 ±3.7 | 55.4 ±3.4 |
| $P_{E_{O_2}}$, mmHg | 126 ±4 | 566 ±38 | 553 ±52 | 536 ±34 | 580 ±40 |
| $P_{E_{CO_2}}$, mmHg | 20.0 ±1.1 | 25.6 ±2.1 | 28.1 ±1.7 | 28.9 ±2.0 | 28.9 ±3.1 |
| $P(ET\text{-}a)_{O_2}$, mmHg | 37 ±11 | 218 ±70 | 169 ±38 | 185 ±74 | 178 ±65 |
| $P(a\text{-}ET)_{CO_2}$, mmHg | 3.3 ±2.7 | −0.7 ±6.3 | −3.1 ±5.7 | −4.8 ±4.8 | −5.4 ±8.9 |

Values are means ± SD; $n = 5$.

at 30 min after the beginning of liquid ventilation. Mean $Pa_{CO_2}$, in *group A* increased from 29.7 mmHg before to 42.6 mmHg after the first 10 min of liquid ventilation, but there was no further significant increase during the remaining 50 min of liquid ventilation.

In *group B* at the end of the apnea period just prior to the beginning of liquid ventilation, the mean $Pa_{O_2}$ was 132 ± 92 mmHg, the mean $Pa_{CO_2}$ was 52.6 ± 2.6 mmHg, and the mean $pH_a$ was 7.27 ± 0.02. During the first 10 min of liquid ventilation, the mean $Pa_{CO_2}$ decreased to 44.8 mmHg and then increased steadily during the remaining 35 min of liquid ventilation. However, the mean $Pa_{CO_2}$ after 45 min of liquid ventilation was not significantly greater than the mean $Pa_{CO_2}$ after 10 min of liquid ventilation.

There were no significant changes during liquid ventilation in $P_{ET_{CO_2}}$, $P_{ET_{O_2}}$ or $P_{E_{O_2}}$ in *group A*. However, in *group B*, $P_{ET_{CO_2}}$ after 45 min of liquid ventilation was significantly greater ($P \leq 0.0025$) than $P_{ET_{CO_2}}$ after 10 min of liquid ventilation.

Mean $Pa_{CO_2}$ in *group A* was consistently lower than in *group B*, although the differences were not significant (10 min, $P = 0.59$; 20 min, $P = 0.66$; 30 min, $P = 0.06$; 45 min, $P = 0.09$).

The $pH_a$ in both groups decreased significantly ($P = 0.009$) during the first 10 min of liquid ventilation, but remained stable thereafter.

The differences between $Pa_{CO_2}$ and $P_{ET_{CO_2}}$, $P(a\text{-}ET)_{CO_2}$, became negative in both groups during liquid ventilation; although in *group A*, $P(a\text{-}ET)_{CO_2}$ was significantly less than 0 ($P \leq 0.02$) only after 30 and 45 min of liquid ventilation, and in *group B*, the $P(a\text{-}ET)_{CO_2}$ was significantly less than 0 ($P = 0.02$) only after 30 min of liquid ventilation. There was no significant difference between $P(a\text{-}ET)_{CO_2}$ in *group A* and *group B*.

The effective alveolar ventilation, $\dot{V}_{A_e}$, was calculated using the following equation:

$$\dot{V}_{A_e} = f\,(V_T - V_{D_{physiol}})$$

There was no significant difference in $\dot{V}_{A_e}$ between *group A* and *group B* during either ventilation with room air or with FC-80 fluorocarbon, although the $\dot{V}_{A_e}$ in *group B* was less than in *group A*, except after 45 min of liquid ventilation.

Anatomical dead space, $V_{D_{anat}}$, was calculated using the Bohr equation (Bohr 1891), and physiological dead space, $V_{D_{physiol}}$, was calculated using the Enghoff (1938) modification of the Bohr equation; the results are given in Table 3. $V_{D_{anat}}$ did not change significantly during either air or liquid ventilation in either group, nor was there a significant difference between $V_{D_{anat}}$ in *group A* and $V_{D_{anat}}$ in *group B*. In both groups, $V_{D_{physiol}}$ during the control period was significantly greater ($P = 0.05$) than $V_{D_{anat}}$. However, this pattern was reversed during liquid ventilation. $V_{D_{physiol}}$ was consistently less than $V_{D_{anat}}$ in both groups, even though the difference was significant ($P = 0.03$) only in *group A* after 45 min of liquid ventilation.

Heart rates increased during liquid ventilation in both groups, but the differences were not statistically significant. In general, the inspiratory and expiratory femoral arterial systolic pressures were greater during liquid ventilation than during air breathing in both groups. The

**TABLE 3**
ANATOMICAL AND PHYSIOLOGICAL DEAD SPACE

| | | Ventilation with FC-80 Fluorocarbon, Elapsed Time, min | | | | |
|---|---|---|---|---|---|---|
| | Air | 10 | 20 | 30 | 45 | 60 |
| | | *Group A* | | | | |
| $V_{D_{anat}}$, ml | 88 ±27 | 95 ±38 | 96 ±22 | 108 ±24 | 105 ±18 | 99 ±22 |
| $V_{D_{physiol}}$, ml | 105 ±21 | 85 ±20 | 87 ±17 | 90 ±35 | 72 ±29 | 84 ±14 |
| | | *Group B* | | | | |
| $V_{D_{anat}}$, ml | 85 ±11 | 87 ±14 | 87 ±15 | 92 ± 7 | 95 ±10 | |
| $V_{D_{physiol}}$, ml | 104 ±13 | 84 ±21 | 77 ±17 | 86 ±12 | 84 ±11 | |

Values are means ± SD; $n = 5$.

same trend was noticed in the inspiratory and expiratory pulse pressures.

The results obtained from the dogs in *group C* are given in Table 4. The measurements made 100, 140, 165, and 180 min after the onset of anesthesia correspond approximately with the measurements made at the following times in the liquid-breathing dogs: air control, 20, 45, and 60 min after the beginning of liquid ventilation. There were no statistically significant changes in any of the parameters from 100 to 180 min after the onset of anesthesia in *group C*.

**Histological findings**

Scanning electron microscopic examination of lungs from the control dogs showed an essentially normal ultrastructural appearance. Cilia in the larger airways were intact. Respiratory bronchioles exhibited a normal epithelial lining, and alveoli were lined by a smooth surface punctuated by occasional pores of Kohn. Some pathologic findings were noted in dogs that had been ventilated with FC-80 fluorocarbon. In the lungs from dog No. 3 (killed 24 h after liquid ventilation), there was an increased number of inflammatory cells in the alveoli. On

**TABLE 4**
DATA FROM GROUP C DOGS

| | Time After Onset of Anesthesia, min | | | |
|---|---|---|---|---|
| | 100 | 140 | 165 | 180 |
| f, breaths/min | 13.3 ±0.3 | 13.3 ±0.3 | 13.3 ±0.3 | 13.3 ±0.3 |
| $\dot{V}_E$, ml(BTPS)/min | 3830 ±83 | 3830 ±83 | 3830 ±83 | 3830 ±83 |
| $\dot{V}_{A_e}$, ml(BTPS)/min | 2450 ±122 | 2382 ±177 | 2481 ±228 | 2439 ± 15 |
| $PaO_2$, mmHg | 102 ± 1 | 101 ± 1 | 101 ± 2 | 104 ± 1 |
| $PaCO_2$, mmHg | 31.7 ±1.8 | 30.7 ±3.5 | 29.4 ±2.6 | 29.8 ±2.8 |
| $pH_a$ | 7.42 ±.02 | 7.44 ±.02 | 7.44 ±.02 | 7.44 ±.02 |
| Base Excess, mEq/liter | −2 ±1 | −2 ±1 | −2 ±1 | −2 ±1 |
| $\dot{V}_{D_{physiol}}$, ml | 104 ± 5 | 110 ± 8 | 102 ±15 | 103 ± 1 |
| $\dot{V}_{O_2}$, ml(STPD)/min | 78 ±9 | 74 ±4 | 75 ±6 | 74 ±8 |
| $\dot{V}_{CO_2}$, ml(STPD)/min | 86 ±3 | 82 ±3 | 81 ±1 | 80 ±8 |
| R | 1.12 ±.16 | 1.11 ±.10 | 1.10 ±.07 | 1.10 ±.23 |

Values are means ± SD; $n = 3$. Dogs were ventilated with room air for approximately 3 h after initiation of anesthesia.

occasion these inflammatory cells were noted to be situated within pores of Kohn. Some inflammatory cells were also noted in the respiratory bronchioles upon an intact epithelial surface. Lungs from dogs that were killed 10, 30, and 180 days after liquid ventilation exhibited some inflammatory cells (presumably macrophages) in alveoli, but these were not common.

The light microscopic appearance of lungs from the control dog was normal. Dog No. 3 (killed 24 h after liquid ventilation) did show increased numbers of neutrophils, particularly surrounding terminal bronchioles and respiratory bronchioles. The lungs from dogs killed 10, 30, and 180 days after liquid ventilation did not exhibit this neutrophilic response but did show slightly increased numbers of macrophages, especially around alveolar ducts. Also, these lungs frequently exhibited foamy cytoplasm.

### Survival

All dogs survived liquid ventilation. Those dogs which were not killed have been kept in the Laboratory Animal Resources Facilities at Duke University under close observation for more than one year after being ventilated with FC-80 fluorocarbon, and have shown no adverse clinical effects. The dogs which were killed also showed no adverse clinical effects.

## DISCUSSION

In dogs which were mechanically ventilated with hyperbarically oxygenated saline, the $CO_2$ partial pressures in expired liquid after the exhalation of 82–93% of the tidal volume ranged from 28 to 74% of the simultaneously measured $Pa_{CO_2}$ (Kylstra, Paganelli, and Lanphier 1966). In those experiments, the respiratory frequencies ranged from 8 to 12 breaths/min, and there was a clear, although statistically not significant, trend for the end-tidal $P_{CO_2}$ to approach the $Pa_{CO_2}$ as the duration of the respiratory cycle increased. In human lungs ventilated with saline at frequencies between 0.4 to 2.4 breaths/min, no evidence of persistent intra-alveolar gas partial pressure gradients was found (Kylstra, Schoenfisch, Herron, and Blenkarn 1973). We had not expected to find any significant difference between end-tidal and arterial $P_{CO_2}$ in dogs ventilated with oxygenated fluorocarbon liquid at a frequency of 2.8 breaths/min, even though the diffusion coefficient of $CO_2$ in FC-80 is slightly lower than in saline,[1] but we certainly had not expected that the end-tidal $P_{CO_2}$ could be greater than the arterial $P_{CO_2}$.

Other investigators (Hanson, Tabakin, and Levy 1967; Gurtner, Song, and Farhi 1969; Robertson and Hlastala 1976) have reported a negative $P(a\text{-}ET)_{CO_2}$ in air-breathing animals, but the cause thus far remains unknown. The phenomenon, if real, could minimize or mask the presence of incomplete intra-alveolar diffusive mixing. If, for instance, the $CO_2$ pressure in liquid adjacent to the alveolar walls would be higher than in the end-capillary blood, then $P_{ET_{CO_2}}$ could be equal to or greater than $Pa_{CO_2}$ despite the presence of diffusive $CO_2$ partial pressure gradients in the alveoli. The $P(a\text{-}ET)_{CO_2}$ was similar in both groups of dogs despite the different respiratory frequencies. Since incomplete diffusive mixing tends to be more pronounced at higher respiratory frequencies (Kylstra et al. 1966), we conclude that diffusive mixing must have been complete or nearly so at the end of each respiratory cycle in both groups.

As seen in Table 1, there were no significant changes in arterial blood gas partial pressures or in end-tidal or mixed expired fluorocarbon gas partial pressures of the *group A* dogs during

[1] $D_{CO_2}$ in saline at 37°C is $2.55 \times 10^{-5}$ cm²/s (Altman and Dittmer 1971). $D_{CO_2}$ in FC-80 fluorocarbon at 37°C has been estimated using Scheibel's empirical equation (Reid and Sherwood 1966) to be $1.05 \times 10^{-5}$ cm²/s.

the last 30 min of liquid ventilation, indicating that, by that time, a steady state had been established.

The $\dot{V}CO_2$ during liquid ventilation was calculated using the equation $\dot{V}CO_2 = \dot{V}E(PE_{CO_2} - PI_{CO_2}) \times \alpha_{CO_2}$, where $\alpha_{CO_2}$ = the solubility coefficient for $CO_2$ in FC-80 at 37°C. The results are shown in Table 5. When values for $\alpha_{CO_2}$ reported by Sargent and Seffl (1970) or by Tham, Walker, and Modell (1973) are entered in the above equation, the $\dot{V}CO_2$ during liquid ventilation was lower than the $\dot{V}CO_2$ during ventilation with room air. During the last 30 min of liquid ventilation, the dogs in *group A* were in a steady state. Therefore, the $\dot{V}CO_2$ during that time should have been the same as during ventilation with room air, unless there was a decrease in the animal's metabolism after the $\dot{V}CO_2$ measurement while the dogs were ventilated with room air. In three anesthetized dogs ventilated with room air for 3 h, there was no significant change in either $\dot{V}CO_2$ or $\dot{V}O_2$ (Table 4). In this group, the anesthetic dose and the duration of anesthesia were both the same as in the liquid-ventilated dogs. Therefore, it seems unlikely that the calculated decrease in $\dot{V}CO_2$ during liquid ventilation was due to depressed metabolism caused by the anesthetic. It is, of course, possible that the presence of FC-80 in the lungs would have depressed the metabolism of the liquid-ventilated dogs directly or indirectly. However, in *group B* there was no significant difference between $\dot{V}O_2$ during ventilation with room air and during ventilation with FC-80 (Table 6) and, therefore, no evidence of depressed metabolism. In *group A*, there was a significant ($P = 0.04$) decrease in $\dot{V}O_2$ during liquid ventilation compared to $\dot{V}O_2$ during ventilation with room air, but this probably was caused by measurement errors. These five experiments were the first of the 13 reported here. The $\dot{V}CO_2$ and $pH_a$ of *groups A* and *B* were the same during ventilation with room air. Furthermore, the

**TABLE 5**
CARBON DIOXIDE PRODUCTION

| $\alpha CO_2$ ($mlCO_2$/literFC-80/mmHg) | Air | Ventilation with FC-80 Fluorocarbon, Elapsed Time, min | | | | |
|---|---|---|---|---|---|---|
| | | 10 | 20 | 30 | 45 | 60 |
| | | *Group A* | | | | |
| 2.1 (Sargent and Seffl) | 74 ± 9 | 43 ± 5 | 45 ± 8 | 47 ± 9 | 45 ± 9 | 48 ± 6 |
| 2.6 (Tham et al.) | 74 ± 9 | 56 ± 5 | 58 ± 9 | 58 ±11 | 57 ±11 | 59 ± 7 |
| 3.2 (our estimate) | 74 ± 9 | 68 ± 6 | 73 ±10 | 72 ±13 | 70 ±14 | 73 ± 9 |
| | | *Group B* | | | | |
| 2.1 (Sargent and Seffl) | 74 ± 4 | 46 ± 9 | 47 ± 5 | 48 ± 6 | 51 ± 4 | |
| 2.6 (Tham et al.) | 74 ± 4 | 58 ±12 | 59 ± 7 | 61 ± 8 | 64 ± 5 | |
| 3.2 (our estimate) | 74 ± 4 | 71 ±15 | 72 ± 8 | 74 ± 9 | 79 ± 6 | |

Values are means ± SD; $n = 5$ for each group.

**TABLE 6**
OXYGEN CONSUMPTION

| Air | Ventilation with FC-80 Fluorocarbon, Elapsed Time, min | | | | |
|---|---|---|---|---|---|
| | 10 | 20 | 30 | 45 | 60 |
| *Group A* | | | | | |
| 97 ±24 | 75 ±17 | 61 ±10 | 65 ±17 | 60 ±16 | 57 ±17 |
| *Group B* | | | | | |
| 85 ±20 | 79 ±22 | 82 ±34 | 92 ±27 | 70 ±23 | |

Values are means ± SD; $n = 5$ for each group.

$\dot{V}_{O_2}$ calculated during air ventilation in *group A* was unusually large compared to $\dot{V}_{O_2}$ in *groups B* and *C* (97 versus 85 and 78 ml (STPD)/min, respectively). An error of ± 3 mmHg in a $P_{E_{O_2}}$ of 123 mmHg measured during ventilation with room air would result in a ± 15 ml(STPD)/min error in the calculated $\dot{V}_{O_2}$. Mauderly (1974) reported a mean $\dot{V}_{O_2}$ of 95 ml (STPD)/min in 30 male beagle dogs with a mean weight of 10.1 kg, but these animals were not anesthetized and paralyzed.

Since the apparent decrease in $\dot{V}_{CO_2}$ in *groups A* and *B* seems not to have been caused by a change in the metabolism of the dogs, an alternate possibility, i.e., that the reported solubility coefficients for $CO_2$ in FC-80 are both too low, needs to be considered. The solubility coefficient for $CO_2$ in FC-80 at 37°C reported by Tham et al. (1973) is 24% larger than the value reported by Sargent and Seffl (1970). Considering the technical problems involved in these measurements (compare Technical information, 3M Co.) it would seem reasonable to consider the possibility that neither of these reported values is correct. A steady state had been achieved by the dogs in *group A* during the last 15 min of liquid ventilation; therefore, the $\dot{V}_{CO_2}$ during that time can be considered equal to the value calculated during the control period. Hence, $\alpha_{CO_2}$ can be calculated from the $\dot{V}_E$, $P_{I_{CO_2}}$, and $P_{E_{CO_2}}$ measured 45 to 60 min after the beginning of liquid ventilation in *group A*. This yields a value of 3.2 ml/liter/mmHg, which is 23% greater than the value reported by Tham et al. (1973).

It does not appear that the dogs in *group B* ever achieved a steady state during 45 min of liquid ventilation. There was a significant increase in $P_{ET_{CO_2}}$ during liquid ventilation, and a trend (though not statistically significant) for an increase in $Pa_{CO_2}$ which was absent in the dogs in *group A*. The $\dot{V}_{A_e}$ was greater in *group A* than in *group B* (though the differences were not statistically significant) and this probably accounts for the lower $Pa_{CO_2}$ in *group A*.

In the animals of *group A*, the $Pa_{CO_2}$ remained constant at a near normal level (Mauderly 1974) throughout 60 min of liquid ventilation at a normal body temperature. However, the $pH_a$ during the period was lower than normal. Before ventilation with liquid, the same animals had a lower than normal $Pa_{CO_2}$ at a normal $pH_a$, indicating the presence of a compensated metabolic acidosis, probably caused by the 24-h fast before the experiment. During the period of liquid ventilation, the $Pa_{CO_2}$ increased to a near normal level: the pre-existing metabolic acidosis was no longer compensated and the $pH_a$ decreased. There was no significant difference between the base deficit before and during liquid ventilation. Hence it seems reason-

able to conclude that respiratory and acid-base homeostasis would have been maintained were it not for the pre-existing metabolic acidosis.

Scanning electron microscopy confirmed the impression gained from light microscopic examination that only minor pathologic changes occurred after liquid ventilation. Pores of Kohn and epithelial surfaces appeared intact. The only exception to the normal appearance was the inflammatory cells noted in the lungs from the dog killed one day after liquid ventilation. Light microscopy revealed that these cells were neutrophils (neutrophils cannot reliably be distinguished from macrophages by scanning electron microscopy). These findings are, in general, in agreement with the ones reported by Modell, Hood, Kuck, and Ruiz (1971).

In summary we conclude that:

1) It is possible to maintain a steady state of gas exchange and a normal or near normal $Pa_{CO_2}$ at normal body temperature in anesthetized liquid-ventilated dogs.

2) There was no evidence of metabolic acidosis as a result of liquid ventilation.

3) The solubility of $CO_2$ in FC-80 fluorocarbon may be greater than previously reported.

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Matthews, W. H., R. H. Balzer, J. D. Shelburne, P. C. Pratt, and J. A. Kylstra. 1978. Échange gazeux continu chez le chien normothermique anesthesie et ventile a liquide. Undersea Biomed. Res. 5(4): 341–354.—Nous avons fait ventiler des beagles de race pure, anesthésiés et paralysés, à un liquide fluorocarbone FC-80 à 38°C ($P_{I_{O_2}}$ = 685 mmHg) pendant 45 min (5 chiens) ou 60 min (5 chiens). La $Pa_{CO_2}$ a été maintenue à 43 mmHg environ pendant 60 min de ventilation à liquide (volume respiratoire, 290 ml; fréquence respiratoire moyenne, 2,8 respirations/min). La $P_{CO_2}$ à la fin de la respiration dépassait chaque fois la $Pa_{CO_2}$ pendant la ventilation à liquide. Les examens de microscopie optique et de microscopie électronique à balayage n'ont revelé aucune lésion pulmonaire chez des beagles sacrifiés 10, 30, ou 108 jours après la ventilation à liquide, sauf une augmentation légère des monocytes autour des canaux alvéolaires. Nous concluons que l'échange gazeux continu peut être maintenu à des pressions partielles de dioxyde de carbone à peu près normales chez le chien ventilé à FC-80 à une fréquence de 3 respirations/min.

ventilation à liquide
échange gazeux
$P(a - A)CO_2$ négative

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