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Pulmonary Function after Ventilation with Fluorocarbon Liquid (Caroxin-D)

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To determine whether animals can breathe fluorocarbon liquid and then be reconverted to breathing air without permanent alterations in lung function or structure, 32 mongrel dogs were anesthetized and ventilated for an hour with Caroxin-D fluorocarbon liquid. In all animals arterial blood–gas tensions were measured and pH determinations made serially. In 16 dogs, pulmonary resistance, compliance, and V_D/V_T were measured before and repeatedly after liquid ventilation. During ventilation with Caroxin-D, adequate arterial oxygen tensions were maintained; P_{CO_2} increased and pH decreased, returning to normal immediately after reconversion to breathing gas. A decrease in pulmonary compliance was found 24 hours after ventilation with fluorocarbon, but this value returned to normal within 72 hours. Pa_{O_2}'s 24 hours after ventilation with Caroxin-D were significantly higher than in previous studies with FX-80. Surviving animals were followed for at least a year, after which time normal pulmonary function was found. The authors conclude that dogs can be ventilated with Caroxin-D fluorocarbon briefly with return to normal lung function for at least a year, even though residual fluorocarbon remains in the lung after a year. (Key words: Liquid breathing; Fluorocarbon; Pulmonary lavage; Pulmonary function.)

RECENTLY, we demonstrated that adult dogs can be oxygenated by ventilation with a fluorocarbon liquid (FX-80 ¶) at 1 atmosphere. Upon reconversion to breathing gas, ventilation was labored and the dogs were hypoxemic for approximately a week when they breathed air. Histologic studies of the lungs showed acute inflammation three hours after liquid ventilation, following by a macrophagic response which was most prominent at 72 hours. A return toward normal was apparent at ten days. Significant numbers of vacuolated macrophages were still present at one month, but completely disappeared by 18 months.[1]

If man is to breathe fluorocarbon liquid, one which causes no permanent alteration in lung function or structure must be found. We continued, therefore, to seek a more suitable liquid. Since FX-80 is a mixture of at least eight compounds,[2] we chose to study pulmonary function of dogs ventilated with another fluorocarbon liquid, Caroxin-D,** which shows only one major peak on gas chromatography. This should eliminate the possibility that any changes in structure or function of the lungs might be secondary to significant amounts of an impurity or isomer.

Materials and Methods

Thirty-two mongrel dogs (weight 15 ± 3.3 kg ††) were anesthetized with sodium pentobarbital and paralyzed with succinylcholine chloride during one hour of liquid ventilation with Caroxin-D. The same basic methodology described previously was followed.[1] One hour of liquid ventilation was chosen because, if found applicable to man, this would be ap-

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¶ $C_8F_{16}O$. 3M Company, St. Paul, Minn. (vapor pressure at 37 C = 58 mm Hg; boiling point 99.93 C; density 1.88 g/ml).

** $C_{18}F_{36}O_2$. Trademark of Allied Chemical Corporation, Morristown, N. J. (formerly identified as P-1D) (vapor pressure at 37 C = 12.8 mm Hg; boiling point 135 C; density 1.75 g/ml).

†† All values expressed in this manner are means ±SD.

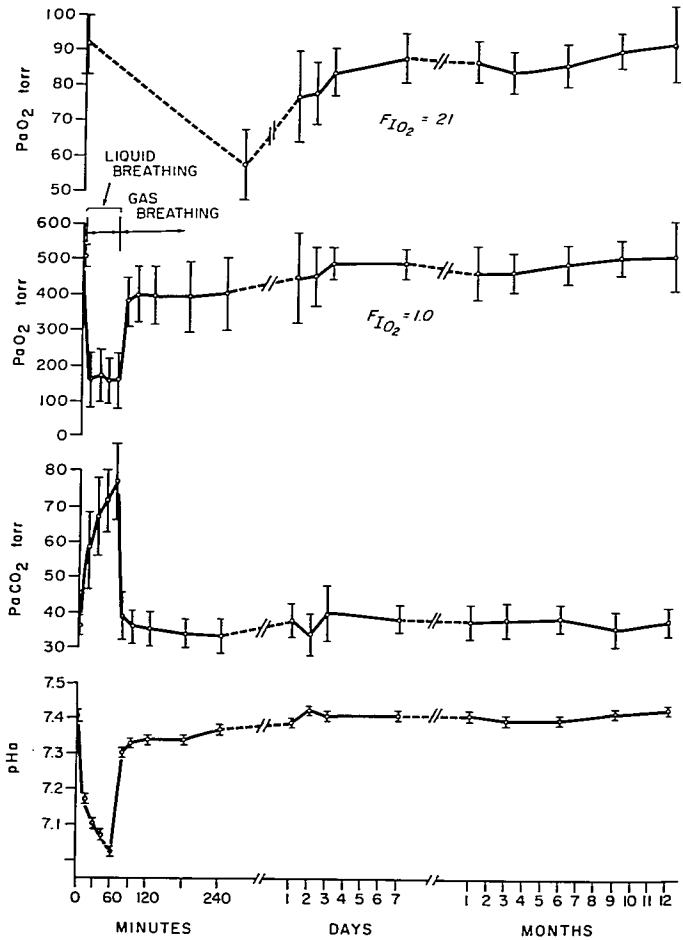


FIG. 1. Arterial blood–gas tensions and *p*H before, during, and for a year after dogs breathed Caroxin-D fluorocarbon liquid for one hour (means ±SD).

TABLE 1. Pulmonary Function before and after Ventilation with Caroxin-D Fluorocarbon

	Control	24 Hr	72 Hr	1 Wk	2 Wk	1 Mo	3 Mo	6 Mo	9 Mo	12 Mo
Number of dogs	16	16	14	14	14	13	13	9	7	8
Compliance (ml·cm H_2O)	15.53 ±10.94	35* ±15.61	42 ±11.52	43 ±10.5	45 ±12.48	44 ±11.4	47 ±20.56	42 ±13.5	50 ±33.5	44 ±13
Resistance (cm H_2O/l/sec)	2.07 ± 1.09	2.51 ± 1.87	2.35 ± 1.40	2.01 ± .92	1.78 ± .69	1.69 ± 1.20	2.60 ± 1.19	3.22* ± 1.30	2.89 ± .92	2.70 ± 1.10
V_D/V_T	.50 ± .05	.48 ± .05	.48 ± .06	.48 ± .01	.48 ± .05	.50 ± .01	.45* ± .07	.45* ± .07	.45* ± .01	.44* ± .005

*$P \leq 0.05$ compared with control value before liquid ventilation.

proximately the time needed for pulmonary lavage. The fluorocarbon was then drained from the lungs by gravity and the dogs returned to breathing gaseous oxygen.

At least three days prior to the experiment, 16 of the dogs were anesthetized with sodium thiopental and their pulmonary resistances, compliances, and deadspace/tidal volume ratios were determined. Pressure, flow, and volume were recorded during controlled ventilation. Compliance was calculated from the relation of volume and pressure at the point of zero air flow at the height of inspiration. Resistance was calculated by choosing two points at equal lung volumes; point A during inspiration and point B during expiration. Since lung volumes are identical at these two points, the pressures overcoming elastic resistance of the lung will be the same. The difference between pressures recorded at points A and B, therefore, represents the sum of the pressures exerted against nonelastic resistance. This pressure (ΔP) was then related to the air flow (F), measured by a pneumotachograph during the same period. Resistance (R) was then obtained by the formula $R = \Delta P/F$.[3] The deadspace/tidal volume ratio (V_D/V_T) was measured using the modified Bohr equation.[4] One, 3, 7, 14, 30, 90, 180, 270, and 360 days after the dogs were ventilated with fluorocarbon liquid, they were reanesthetized with sodium thiopental, 16–20 mg/kg, and pulmonary function studies (PFS) were repeated.

The remaining dogs served as controls and were followed with blood–gas determinations, but without PFS. Nine of the dogs were electively sacrificed for histologic examination of lung tissues five to 20 months after ventilation with fluorocarbon liquid. Their lungs were inflated with 10 per cent buffered formalin at 30 to 50 cm pressure. Samples were taken from each lobe. Nine to 13 pulmonary sections from each animal were examined. All sections were stained with hematoxylin and eosin.

Tissues from Dog 563, sacrificed 20 months after Caroxin-D ventilation, also were analyzed chromatographically for residual fluorocarbon. Known weights (0.2 to 0.6 g) of the tissues (kidney, liver, brain, fat, and lung) were ground, with addition of 4 ml n-Hexane. The resultant solutions were centrifuged, dried and analyzed by injection into a Perkin-Elmer model 990 chromatograph equipped with a Nickel 63 electron capture detector. The carrier gas used was 90 per cent argon and 10 per cent methane at a flow rate of 45 ml/min; the detector was kept at 290 C, operating on pulsed mode at 100-microsecond intervals. A ¼″ × 12′ stainless steel column with 10 per cent silicone DC-200/12,500 on Chromosorb W, AW, DMCS was used. The remaining animals are still alive to permit long-term follow-up studies.

Results

All 32 dogs survived ventilation with Caroxin-D fluorocarbon liquid for one hour and reconversion to breathing gaseous oxygen. The arterial oxygen tension during ventilation with liquid was satisfactory (fig. 1). Pa_{CO_2} increased and pH_a decreased during ventilation with Caroxin-D, but values returned to normal within 15 minutes after the dogs resumed breathing gaseous oxygen (fig. 1). After liquid ventilation, draining by gravity failed to recover 200–300 ml of Caroxin-D from each dog. Although some may have

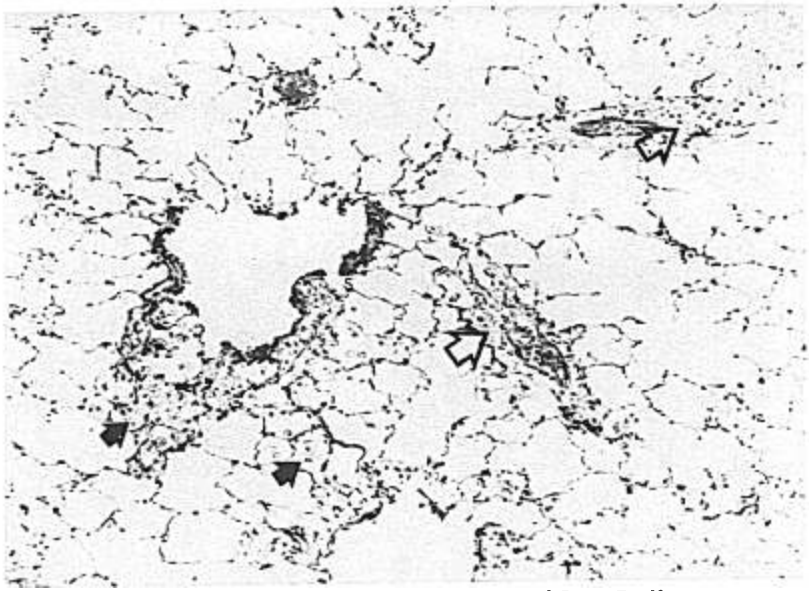


FIG. 2. Lung five months after liquid ventilation with oxygenated Caroxin-D. Note macrophages in alveoli (small solid arrows), particularly around the bronchioles and in the perivascular interstitium (large open arrows) (hematoxylin and eosin, ×135).

evaporated during the experiment, presumably significant amounts remained in the lungs.

Two dogs in the PFS group died 24 hours after liquid ventilation. Examination of their lungs showed pneumonia. Another died of bite wounds four weeks after ventilation with liquid. One was mistakenly sacrificed after survival for a year. A fifth dog died during anesthesia after a year. In the control group two were found dead of bronchopneumonia in their cages three days after liquid ventilation. A third dog pulled out his indwelling arterial catheter on the tenth day and was exsanguinated. Another was found dead a month after liquid ventilation, but autolysis precluded satisfactory postmortem examination. Nine animals were electively sacrificed for examination of the lungs, and the remaining 14 dogs are still alive and well 12 to 18 months after ventilation with liquid.

Since the pH_a, Pa_{CO_2}, and Pa_{O_2} values in the control and PFS groups were almost identical, the two groups were combined for analysis (fig. 1). Pa_{O_2}'s were less than 70 torr ($FI_{O_2} = 0.21$) in only five of the 32 dogs 24 hours after liquid ventilation; within 72 hours they were more than 70 torr in 26 of the 27 survivors. Although the mean Pa_{O_2} at this time was 83 ± 7.2 torr, it was still significantly different from the pre-experimental control value (92 ± 7.7 torr) ($P < 0.001$). Pa_{O_2}'s at $FI_{O_2} = 1.0$ were above 365 torr in all of these dogs at 72 hours. Pa_{O_2}'s reached normal values by seven days and remained normal for the year of study (fig. 1). pH_a's and Pa_{CO_2}'s of the dogs were normal at all times tested after liquid ventilation.

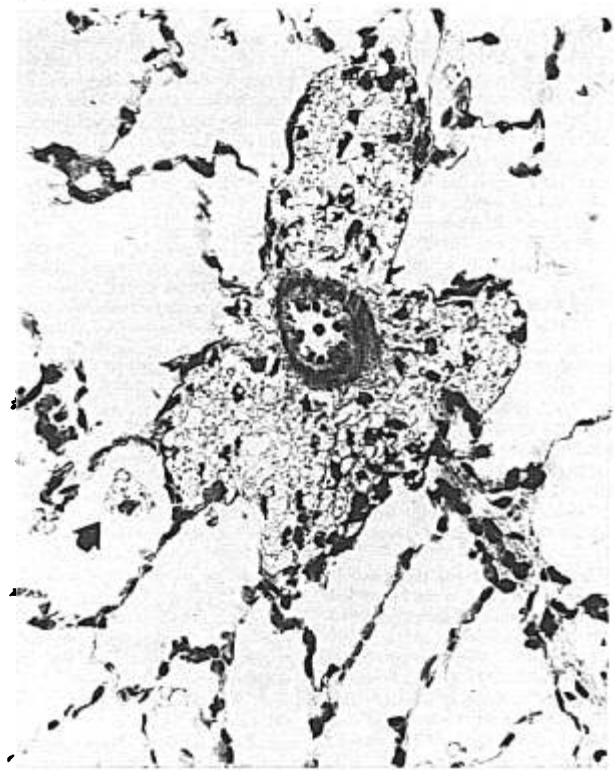
Arterial systolic blood pressure declined approximately 20 torr during inhalation when the lungs were being filled with fluorocarbon,

FIG. 3. *A (above)*, Lung 20 months after liquid ventilation with oxygenated Caroxin-D. Note macrophages clustered around bronchioles and in perivascular interstitium (large open arrows) and the absence of any parenchymal reaction to the macrophages. Some sections of the lung contained far fewer macrophages than are shown in the illustration (hematoxylin and eosin, ×125). *B (facing page)*, Detail of 3A (boxed area) to show vacuolated macrophages in perivascular interstitium, normal alveolar septa, and scattered intra-alveolar macrophages (small solid arrow) (hematoxylin and eosin, ×475).

returning to normal during the exhalation phase in every dog. Intratracheal pressures ranged from 26.1 ± 1.3 torr on inhalation to -22.9 ± 8.9 torr during exhalation.

Decreased lung compliance was found 24 hours after ventilation with liquid ($P = 0.025$). Compliance had returned to normal by one week and, although there was some fluctuation in the mean values for resistance and compliance from time to time, both were normal a year after ventilation with liquid (table 1). The deadspace/tidal volume ratios ranged from 0.5 ± 0.05 to 0.44 ± 0.01 throughout the experiment.

On histologic examination, the lungs of animals sacrificed at intervals from five to seven months after liquid ventilation were indistinguishable from each other. In all lobes of all animals there were varying numbers of large vacuolated macrophages, which presumably contained fluorocarbon (fig. 2). These were scattered singly and in small groups in the alveoli and on the alveolar septa. Larger groups of macrophages formed prominent intra-alveolar clusters around bronchioles. Macrophages were also numerous in the interstitium of the lung, forming sheaths about bronchioles and small vessels, and were present in hilar lymph nodes. Apart from the presence of the macrophages, the alveolar



septa, airways, and vasculature were histologically normal.

The lungs of two animals sacrificed at one year and one animal sacrificed at 20 months (fig. 3) all contained macrophages similar in number and distribution to those in the animals sacrificed at five to seven months. Owing to variation in numbers of macrophages from animal to animal in the dogs sacrificed at five to seven months, it was not possible to state categorically that the animals examined at a year and at 20 months had significantly reduced numbers of macrophages. However, there was no evidence of parenchymal reaction to the residual (fluorocarbon) macrophages.

The extracts from brain, kidney, and liver did not give Caroxin-D peaks even when tested at maximum sensitivity (attenuation 200), and when 5-μl amounts were injected. The extract of fat tissue had a Caroxin-D concentration of 0.0086 mg/g; that of the left lung, 1.58 mg/g; that of the right lung, 1.64 mg/g.

Discussion

This study confirms our earlier reports that life can be supported for brief periods by ven-

tilation with fluorocarbon liquid.[1, 5] Although the dogs in this series did have moderate hypoxemia at $F_{I_{O_2}} = 0.21$, mean Pa_{O_2} was 76 torr at 24 hours and 83 torr three days after liquid ventilation. Both of these values are significantly higher than those found after ventilation with FX-80 ($P < 0.001$). While it took only three to seven days for Pa_{O_2} to return to normal after ventilation with Caroxin-D, it took ten days for a similar return to normal after ventilation with FX-80.[1]

Although mean Pa_{O_2} was 76 torr a day after ventilation with Caroxin-D when the dogs breathed 21 per cent oxygen, it was more than 400 torr when they breathed 100 per cent oxygen. The latter value indicates that a true, or absolute, intrapulmonary shunt due to perfused but nonventilated alveoli played only a minor role in the mild hypoxemia observed while the dogs breathed air. This fact, coupled with the decreased compliance observed at that time, suggests that uneven ventilation might have been responsible for the hypoxemia. Since diffusion studies were not performed, however, we cannot rule out a diffusion problem as a possible contributing factor.

Two other explanations are also possible. First, fluorocarbon remained in the lung. This could restrict the volume of air necessary to fill the lung and, thus, could be reflected in decreased compliance. Even though oxygen is quite soluble in Caroxin-D (42.2 vol per cent at 1 atm gas pressure at 37 C),[6] when the dog breathed room air the residual fluorocarbon would contain only ½ of this amount. The diffusion of oxygen in this liquid, while probably faster than diffusion in saline solution, should be slower than in an equal volume of gas. This may then manifest as a relative shunt, which is minimized when the fluorocarbon is fully saturated with oxygen at a high P_{O_2}.

Furthermore, the vapor pressure exerted by this liquid (12.8 torr at 37 C) would act as a space-occupying lesion and decrease the $P_{A_{O_2}}$ available for transfer. On this basis, one would expect a lower Pa_{O_2} after breathing FX-80 (vapor pressure = 58 torr at 37 C) than after breathing Caroxin-D.

In light of the normal blood gas, compliance, resistance and V_D/V_T values found a year after ventilation with Caroxin-D, we were surprised to find significant amounts of fluorocarbon retained in the lungs when the dogs were sacrificed. Whether this would eventually lead to scarring of the lung tissue and interfere with normal gas exchange awaits further study, although no reactive process, apart from persistence of macrophages, can be demonstrated after 20 months. Apparently, more fluorocarbon was retained after 18 months than was seen in our previous study with FX-80.[1] We attribute this to the lower vapor pressure of Caroxin-D compared with FX-80, which would suggest that the former evaporates more slowly from the lung.

Since pulmonary function tests and Pa_{O_2} returned to normal within three to seven days after liquid breathing, we conclude that dogs can be ventilated with Caroxin-D fluorocarbon liquid with return to normal lung function for at least 20 months, even though residual fluorocarbon remains in the lung.

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