Anesthesiology
V 39, No 5, Nov 1973

Morphologic and Biochemical Changes in Dogs after Ventilation with Caroxin-D Fluorocarbon

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Eleven beagle dogs were ventilated with liquid fluorocarbon Caroxin-D for one hour, then reconverted to breathing gas. A series of hematologic and biochemical tests was performed repeatedly for a year. The animals then were sacrificed; tissues were examined microscopically and were analyzed by gas chromatography for fluorocarbon content. No permanent toxicologic effect was found during the experimental period. Transient elevations in SGOT, SGPT, SAP, and leukocyte counts were present for approximately a week, then returned to normal. Some residual fluorocarbon was present in all tissues examined, but no pathologic change could be attributed to its presence, except for local accumulations of vacuolated macrophages in the lungs and regional lymph nodes. (Key words: Fluorocarbon; Caroxin-D; Liquid ventilation; Pulmonary lavage; Toxicology; Gas chromatography; Beagle.)

We PREVIOUSLY INVESTIGATED the effects of ventilation with oxygenated liquid fluorocarbons on pulmonary mechanics and gas exchange.[1-6] If pulmonary lavage with fluorocarbon liquids is to have clinical application, one must know whether biochemical or morphologic changes follow with its use. This experiment was designed to screen beagle dogs for residual fluorocarbon and for morphologic, biochemical and/or histologic evidence of toxicity after liquid ventilation with Caroxin-D ** fluorocarbon.

Materials and Methods

Eleven adult beagle dogs adjusted to their new environment for a month. Three baseline samples of blood were drawn from the jugular vein at one-week intervals. Each animal then was anesthetized with sodium pentobarbital, 25 mg/kg, and a zero-time blood sample was obtained. The trachea was intubated with a cuffed endotracheal tube and the lungs were ventilated with oxygenated liquid fluorocarbon Caroxin-D, as described previously.[4] All animals were ventilated with liquid fluorocarbon for one hour and then reconverted to breathing 100 per cent gaseous oxygen for three hours. The tracheas were then extubated and the dogs kept in an oxygen tent ($F_{I_{O_2}}$ approximately 0.40) until Pa_{O_2} was more than 70 torr at $F_{I_{O_2}} = 0.21$. Blood was drawn from the jugular vein 1, 3, 7, 14, and 28 days, and 2, 3, 4, 5, 6, 9, and 12 months after ventilation with liquid fluorocarbon. The following measurements were made.

Concentrations of serum sodium and potassium were determined with a flame photometer and serum chloride concentration with a Buchler-Cotlove chloridometer. Serum calcium and magnesium concentrations were measured with an atomic absorption analyzer. Serum alkaline phosphatase (SAP) was deter-

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Received from the Departments of Anesthesiology, Pathology, and Medicine, the University of Florida College of Medicine, Gainesville, Florida 32601. Accepted for publication May 2, 1973. Supported in part by grants from the National Institutes of Health GM17246-04 and from Allied Chemical Corporation.
The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.
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mined using thymolphthalein monophosphate as the substrate.[a] Colorimetric determination for lactic dehydrogenase (LDH) was based on a modification of the technique reported by P. G. Cabaud and F. Wroblewski.[b] A modified Reitman-Frankel[c] colorimetric method was used to determine serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT).

Serum total protein value was determined by the biuret method. Serum globulin was determined by using copper in a mixture of sulfuric and acetic acid reagent.[d]

The method of Ducci and Watson was used to obtain bilirubin values.[7] Blood urea nitrogen (BUN) values were obtained using a method based upon the Fearon Reaction.[e] A modified Lieberman-Burchard reagent[f] was used to determine cholesterol values. Serum uric acid was determined by the reduction of phosphotungstic acid by uric acid in an alkaline solution.[g]

Leukocyte counts were performed on a Coulter Counter calibrated for dog blood. Whole-blood hemoglobin was measured using the cyanmethemoglobin method, and hematocrit with the microcapillary technique. Platelet counts were performed by direct count using Unopette[h] vials for diluting samples. Differential leukocyte and reticulocyte counts were also determined. Plasma hemoglobin was determined by the method of Shinowara.[8] Plasma prothrombin times were run using an Ames Lab Tek Prothrombin System.[i]

After 12 months the dogs were sacrificed. Complete postmortem examinations were made. The lungs and trachea were removed *en bloc* and photographed. One lobe of lung was removed for gas chromatographic study. The lungs then were inflated with 10 per cent buffered formalin at 30 to 50 cm pressure and fixed for two or more days prior to sectioning. Samples for histologic examination were taken from each remaining lobe. All lobes were inspected, and if any anomaly was suspected that area was included in the tissue samples.

Portions of the tracheobronchial lymph nodes, heart, liver, spleen, kidney, and brain also were fixed in 10 per cent buffered formalin for subsequent histologic examination. As many as 11 sections of lung from each animal were examined. All sections were stained with hematoxylin and eosin.

One lobe of the lung and portions of tracheobronchial lymph nodes, subcutaneous and abdominal fat, liver, kidney, spleen, heart, skeletal muscle, and brain also were examined by gas chromatography for residual fluorocarbon as described below. Two to 5 g of each organ tissue were weighed and homogenized with 20 ml hexane. The solution was dried by addition of Drierite[j] and shaken. Depending on the concentration of the solution, 0.4 to 5 μl of extract were injected into a Perkin-Elmer Model 990 Gas Chromatograph equipped with a Ni 63 electron capture detector. A 1/4" × 12' stainless steel tubing packed with 10 per cent silicone DC-200/12,500 on Chromosorb W, AW, DMCS was used. The chromatographic conditions were: column temperature 60 C, injector temperature 100 C, manifold temperature 150 C, detector temperature 300 C, and carrier gas flow rate 45 ml/min. The carrier gas used was a mixture of 10 per cent methane and 90 per cent argon. The detector was operated in the pulsed mode with a pulse interval of 10 microseconds. The fluorocarbon concentrations were calculated from the area of the chromatographic peaks.

Results

Mean serum sodium, chloride, calcium, and magnesium were normal at all time periods. Although the mean serum potassium level was slightly lower than that reported for normal dogs at zero time (3.9 ± 0.3 mEq/l),[††] it returned to normal after ventilation with fluorocarbon. No change in total protein, albumin,

[a] Hycel, Inc., Houston, Texas (KIT B5310-1).
[b] Dade Reagents, Inc., Miami, Florida (KIT B5315).
[c] Dade Reagents, Inc., Miami, Florida (KITS B5323 and B5325).
[d] Hycel, Inc., Houston, Texas (Hycel Reagent 197).
[e] Hycel, Inc., Houston, Texas (Reagents 286, 287, and 288).
[f] Hycel, Inc., Houston, Texas (Reagents 181, 179).
[g] Hycel, Inc., Houston, Texas (Reagents 146, 147, and 148).
[h] Becton Dickinson Co., Rutherford, N. J.
[i] Ames Lab Tek, Inc., Westmont, Ill.

[j] W. A. Hammond Drierite Co., Xenia, Ohio.
†† Mean ± SD.

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TABLE 1. Serum Enzyme Levels after Ventilation with Liquid Fluorocarbon Caroxin-D

	Dog	Pre-fluorocarbon Control Range	Time after Liquid Ventilation											
			24 hr	72 hr	1 wk	2 wk	4 wk	2 mo	3 mo	4 mo	5 mo	6 mo	9 mo	12 mo
SGOT, international units	616	17–28	80	10	25	30	20	27	29	27	23	33	40	43
	617	15–21	35	49	†	26	14	34	24	25	18	25	35	‡
	618	5–33	105	17	32	25	17	34	20	29	18	33	40	41
	619	28–33	54	25	40	27	31	45	26	32	30	39	45	48
	620	22–33	55	*										
	621	21–38	75	85	35	35	27	31	23	34	37	45	40	46
	622	27–43	100	39	54	36	28	33	62	27	35	44	37	45
	623	25–37	71	43	40	37	15	27	37	25	42	37	35	40
	624	21–26	90	42	47	27	12	30	30	24	26	33	32	35
	625	25–67	55	36	23	30	25	38	37	33	33	49	55	40
	627	23–45	54	282	32	35	24	25	30	23	22	33	30	31
	Mean	29	70	63	36	31	21	32	32	28	28	37	39	41
	± SD	±10	±22	±80	±10	±5	±7	±6	±12	±4	±8	±7	±7	±5
SGPT, international units	616	15–26	56	45	11	22	22	36	34	29	31	61	64	57
	617	23–35	51	85	†	18	2	138	52	39	40	40	37	‡
	618	26–45	58	52	18	0	126	90	34	30	28	51	46	40
	619	30–51	46	66	40	38	56	120	49	39	41	46	56	90
	620	16–30	46	*										
	621	18–31	40	63	62	18	14	11	23	24	41	31	56	40
	622	22–42	59	58	29	25	25	54	53	33	70	49	46	38
	623	26–40	52	58	39	56	34	41	44	26	108	44	46	44
	624	16–32	64	65	92	46	31	31	36	29	52	26	35	38
	625	30–57	70	55	42	38	45	65	68	60	67	70	66	53
	627	21–45	96	231	116	51	26	46	53	50	44	52	43	46
	Mean	31	58	78	50	31	38	63	45	36	52	47	50	50
	± SD	±10	±15	±55	±35	±17	±34	±41	±13	±11	±24	±13	±11	±17

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TABLE 1.—(*Continued*)

	Dog	Pre-fluorocarbon Control Range	Time after Liquid Ventilation											
			24 hr	72 hr	1 wk	2 wk	4 wk	2 mo	3 mo	4 mo	5 mo	6 mo	9 mo	12 mo
Alkaline phosphatase, King-Armstrong units	616	4.2–5.9	20.4	24.3	10.5	6.2	4.9	6.6	3.3	4.6	3.6	3.7	2.8	2.6
	617	2.9–6.4	17.8	26.0	†	6.2	2.2	15.0	2.1	2.5	1.6	2.2	4.3	‡
	618	4.6–6.0	14.7	14.0	9.5	5.5	2.8	9.2	3.0	4.4	2.5	2.6	3.1	2.6
	619	5.3–8.9	11.8	9.2	7.5	5.7	4.6	6.4	4.6	5.7	4.0	3.7	3.3	3.1
	620	4.6–5.4	15.9	*										
	621	3.3–7.5	14.2	17.4	8.8	5.6	3.8	6.3	3.1	4.5	3.2	3.7	2.8	2.7
	622	2.8–5.3	10.8	9.2	4.9	3.3	2.4	3.9	4.4	2.0	2.5	2.3	2.9	2.6
	623	2.7–5.4	10.6	9.0	5.0	3.8	2.4	3.2	5.2	2.9	2.3	2.5	3.0	2.3
	624	3.4–5.5	6.9	7.2	4.6	3.2	2.3	4.1	5.9	2.5	2.3	3.0	2.8	3.3
	625	3.0–6.2	8.1	6.1	4.6	3.7	3.3	5.4	8.6	2.4	3.3	3.4	3.4	3.0
	627	3.0–4.8	11.8	9.3	5.8	4.5	5.8	3.9	6.6	2.8	3.3	3.6	2.8	5.2
	Mean	4.7	13	13.2	6.8	4.8	3.4	6.4	4.7	3.4	2.9	3.1	3.1	3.0
	± SD	±1.4	±4.1	±7.1	±2.3	±1.2	±1.3	±3.5	±2	±1.3	±0.7	±0.6	±0.5	±0.9

* Dog died with pneumonia. † No sample drawn. ‡ Dog died during cesarean section.

globulin, total bilirubin, BUN, or uric acid level was seen after liquid ventilation. There was a transient increase in cholesterol 24 to 72 hours following liquid ventilation (223 ± 48 and 287 ± 88 mg/100 ml, respectively) but it rapidly returned to baseline values.

SGOT was above the normal range in six dogs 24 hours after liquid ventilation ($P < 0.001$). However, it had returned to normal in four of these animals by 72 hours, and in the remaining two dogs by a week. Alkaline phosphatase and SGPT were elevated 24 to 72 hours after liquid ventilation ($P < 0.001$) but returned to normal in all dogs shortly thereafter. While isolated increases in SGPT and alkaline phosphatase were occasionally seen one to 12 months later, no dog showed a consistent abnormality from one time period to the next (table 1). Mean LDH was normal throughout; however, two dogs had transient elevations at one week.

Hemoglobin and hematocrit values were normal throughout the experiment. There was a significant decrease in the mean platelet count 24 hours after liquid ventilation from $4.22 \pm 0.83 \times 10^5$ to $2.85 \pm 0.44 \times 10^5$ ($P < 0.001$); however, this value is still within normal limits. The prothrombin time did not change from the control value during the experiment. The baseline leukocyte count was moderately elevated (18.3×10^3). A significant drop was seen at 4 weeks ($P < 0.01$); however, the mean count of 11,000 was still well within the normal range. Immature neutrophils increased at 24 hours, suggesting that an inflammatory process was present.

Four dogs developed clinical evidence of pneumonia that necessitated oxygen and antibiotic therapy for 1 to 14 days. Of these, one died at 24 hours. Postmortem examination revealed acute hemorrhagic pneumonia and focal acute tubular necrosis of the kidneys. One dog died at 9 months during cesarean section.

At postmortem examination the lungs of the nine animals sacrificed at 12 months were fully expanded, pink, crepitant, and soft without nodularity. There was no gross evidence of emphysema or fibrosis.

On histologic examination the lungs of the animals were indistinguishable from one another and were similar to those described previously.[3] All sections of all lungs contained

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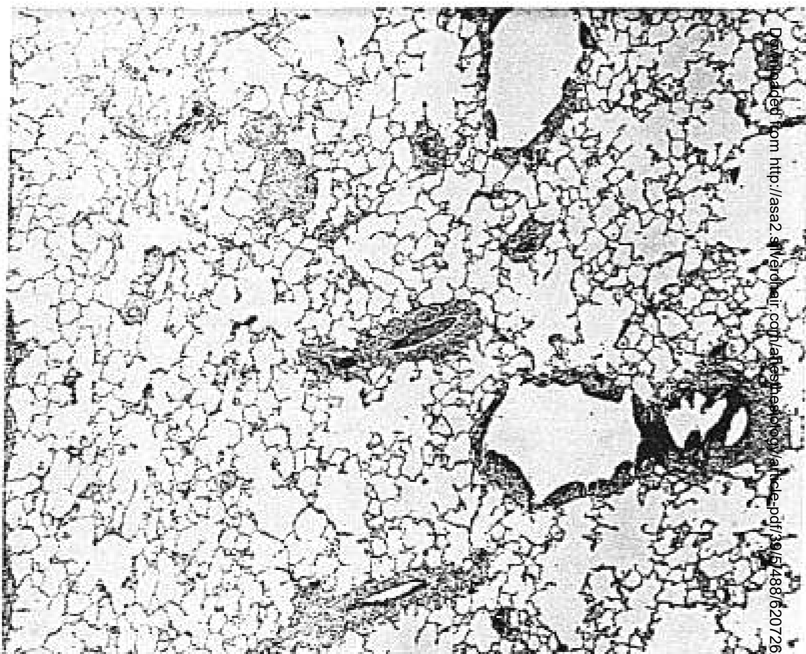

FIG. 1A. Lung 12 months after ventilation with Caroxin-D fluorocarbon. Note predominantly perivascular, peribronchial and subpleural accumulations of macrophages. The pattern of alveolar ducts and alveoli is normal, and there is no evidence of emphysema (hematoxylin and eosin, ×35).

many finely vacuolated macrophages aggregated linearly beneath the pleura and in prominent cuffs around bronchi, bronchioles, and blood vessels. Most of these macrophages were in the interstitium, while fewer macrophages filled the contiguous alveoli (fig. 1, *A* and *B*). In addition, scattered vacuolated cells were attached to alveolar septa, while others lay free in the alveoli. Some macrophages appeared to be degenerating, as evidenced by their fragmented karyorrhectic nuclei. In a few sections from some animals there were small parabronchial collections of lymphocytes with a light, variable admixture of plasma cells. These were generally located in the cuffs of macrophages, but did occur around some larger bronchi independent of and separate from the macrophage reaction. In no section was there any evidence of reactive fibrosis to the macrophage aggregates. In sections of the tracheobronchial lymph nodes there were large accumulations of identical macrophages in the interstitium (fig. 2). The peripheral sinuses were clear. No significant histologic abnormality was detected in sections of heart, kidney, spleen, liver, and brain.

One to 2 mg of fluorocarbon were present in each gram of lung and lymph node tissue analyzed (table 2). The concentration dropped by a factor of 50 to 100th of this amount in fat and to 1,000th of this amount in brain, spleen, liver, kidney, muscle, and heart (table 2).

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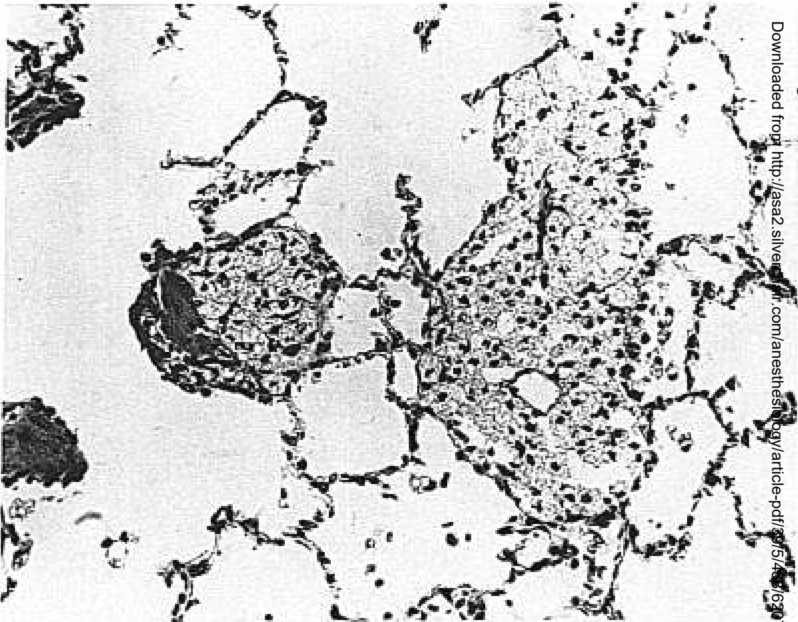

FIG. 1B. Detail of figure 1A, showing perivascular and peribronchial accumulations of macrophages. Note also scattered intra-alveolar macrophages and the normal alveolar septa (hematoxylin and eosin, ×250).

Discussion

No permanent significant biochemical or morphologic change was demonstrated in this study. The only significant abnormal values observed were elevations of SGOT for 24 hours and SGPT and SAP for 72 hours. Elevations of SGOT and SGPT can be indicative of hepatic cellular damage. However, Kaneko and Cornelius question the importance of inconsistent changes in these enzymes, since nutritional and climatic changes can also produce elevations in these enzyme levels in dogs.[9] Since the majority of our animals showed an increased enzyme level 24 to 72 hours after liquid ventilation, it is likely that this resulted from the stress of anesthesia, surgery, and/or liquid ventilation. These animals also had been without food for at least 24 hours, so this cannot be excluded as a possible contributory factor. It is important to emphasize, however, that regardless of the cause, enzyme levels returned to normal in all animals. We ascribe the isolated elevations observed during the 12 months of follow-up study to other factors, as suggested by Kaneko and Cornelius.[9] That the bilirubin level was never elevated strongly supports the conclusion that significant hepatic dysfunction did not occur in this study. Likewise, the total protein, albumin, and globulin levels did not change after ventilation with liquid fluorocarbon. While the pre-experimental albumin level was slightly less than that reported by some authors, it was consistent with values obtained in this laboratory for normal dogs.[4]

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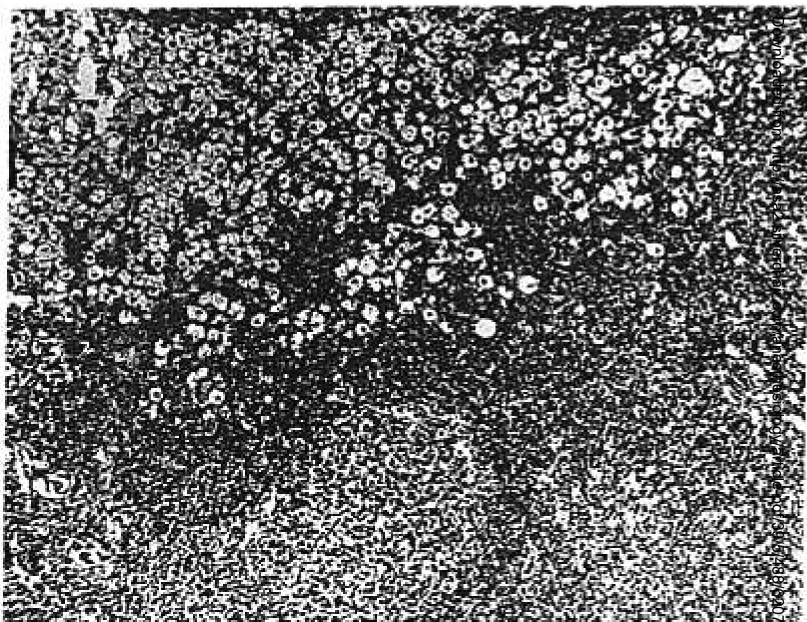

FIG. 2. Tracheobronchial lymph node 12 months after ventilation with Caroxin-D fluorocarbon. Note the numerous pale macrophages in the interstitium of the node in the upper half of the photograph. Portions of two normal lymph follicles are present in the lower half (hematoxylin and eosin, ×145).

The LDH value is derived from a ubiquitous group of isoenzymes found in most organ systems. Their significance as a diagnostic test for a special organ function in the dog is doubtful.[9,10] Elevations as high as 800 units have been reported for normal, exercised beagles.[10] In this study, two dogs had values of more than 1,000 units at one week. One of these had pneumonia, which necessitated oxygen supplementation and antibiotic therapy. Otherwise, the values found in this experiment were within those reported as normal for beagles.[9,10]

The etiology of the transient rise in cholesterol is unknown. One explanation is that the dogs that were anorectic in the post-liquid-ventilation period were usually offered more tempting diets than the standard dry kennel chow. These may have been higher in cholesterol content. Our findings would then agree more closely with those for normal household pets, *i.e.*, 258 ± 36 mg/100 ml.[9]

The hematology values found were compatible with the histologic findings in an earlier report on liquid ventilation, in which there was a generalized acute inflammatory reaction in the lungs four hours after ventilation with liquid fluorocarbon. This was followed at 72 hours by an infiltration of macrophages.[2] The lungs of dogs sacrificed four hours after liquid ventilation were hyperemic, and some contained local intraalveolar hemorrhage. By ten days, the histologic picture no

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longer showed acute changes, and only residual macrophages were found.[3]

In the present study no evidence of pathologic change was seen in the liver, spleen, kidney, heart, or brain. Even the lung, which still contained numerous macrophages and a significant quantity of residual fluorocarbon, was free from irreparable reaction to this material, as evidenced by the total absence of fibrosis from all sections examined. This is consistent with another study in which dogs were sacrificed 20–23 months after ventilation with Caroxin-D fluorocarbon. The lung concentrations at that time were approximately 5 per cent less than after 12 months.[11] Since fluorocarbon is lipophilic and hydrophobic, we were not surprised to see a higher concentration of fluorocarbon in fat than in leaner tissues. The fact that Caroxin-D fluorocarbon is still present in the dog a year after liquid ventilation is of some concern. However, since neither biochemical nor irreparable histologic change secondary to its presence could be demonstrated at that time, we question the importance of the residual fluorocarbon to the well-being of the animal.

In conclusion, we were unable to demonstrate consistent abnormalities in the morphologic and biochemical parameters measured in this study after ventilation with Caroxin-D fluorocarbon. Within 24 to 72 hours there was an increase in enzyme levels, which may represent transient hepatic changes; however, these values rapidly returned to normal. Leukocyte shifts were consistent with an acute inflammatory response, as reported previously. While residual fluorocarbon was present after a year, particularly in the lung and lymph nodes, no detrimental consequence of its presence could be demonstrated.

TABLE 2. Concentrations of Fluorocarbon Caroxin-D in Tissues One Year after Liquid Ventilation (Means ± SD)

Tissue	Concentration (mg Fluorocarbon/g Tissue)
Lung, dorsal	1.763 ± 0.170
Lung, ventral	1.795 ± 0.146
Lymph nodes (tracheobronchial)	1.883 ± 0.179
Brain	0.00284 ± 0.0004
Spleen	0.00243 ± 0.0005
Liver	0.00438 ± 0.0005
Kidney	0.00241 ± 0.0003
Muscle	0.00183 ± 0.0002
Heart	0.00157 ± 0.0001
Fat (subcutaneous)	0.0402 ± 0.0054
Fat (abdominal)	0.0513 ± 0.0037

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