4. D. L. Fox and G. F. Crozier, *Science* **150**, 771 (1965); S. de Prailaune, *Compt. Rend. Soc. Biol.* **149**, 655 (1955); M. Vialli, *Rend. Ist. Lombardo Sci. Pt. I* **91**, 680 (1957).
5. Captured on hook and line on octopus bait by local fisherman Ahmed Hussein from about 150 m depth at midnight, 11 Dec. 1964, about 1 km offshore from Mutsamudu, Island of Anjouan, Comores. Fish 1.37 m long, 35.5 kg fresh weight. Successfully preserved and shipped to Los Angeles through the efforts of Dr. P. Liaume, Mutsamudu. Administrative arrangements made with the assistance of President Said M. Cheikh, Governing Council, Territory of the Comores; Ambassador C. Vaughan Ferguson, Jr., American Embassy, Tananarive, Madagascar; and Dr. John H. Ryther, Woods Hole Oceanographic Institution, Woods Hole, Mass. Support for the operation from NSF via U.S. Program in Biology, International Indian Ocean Expedition.
6. J. C. Nevenzel, W. Rodegker, J. F. Mead, *Biochemistry* **4**, 1589 (1965).
7. G. Rouser, C. Galli, G. Kritchevsky, *J. Am. Oil Chem. Soc.* **42**, 408 (1965).
8. J. Millot, "Le troisième coelacanthe," *Naturaliste Malgache*, Suppl. 1 (Inst. de Recherches Scientifique, République Malgache, 1954).
9. Histological studies of tissue lipid localization done by sectioning formalin-fixed material at 15 μ on freezing microtome and staining with oil red O and hematoxylin [G. L. Humaston, *Animal Tissue Techniques* (Freeman, San Francisco, 1962), p. 254]. Histology of all tissues for light microscopy was performed by L. Meiers, of muscle for the electron microscope, by J. Adomian.
10. The density of oleyl oleate is 0.8600 at 30°C; that of triolein is 0.8988 at 40°C [F. Richter, Ed., *Beilsteins Handbuch der Organischen Chemie*, 3rd suppl. (Springer Verlag, Berlin, ed. 4, 1961), vol. II-2, pp. 1415, 1423].
11. Both from Analytical Engineering Labs, Hamden, Connecticut.
12. Supported by contract AT(04-1GEN-12 between the U.S. Atomic Energy Commission and the University of California; in part by PHS research career award GM-K6-19, 177 to J.F.M. and by NSF grant GB 3584 to M.S.G.

18 April 1966

Survival of Mammals Breathing Organic Liquids Equilibrated with Oxygen at Atmospheric Pressure

Abstract. *Because oxygen and carbon dioxide are very soluble in certain silicone oils and fluorocarbon liquids, these liquids will support respiration of mammals. Mice and cats respiring silicone oil die shortly after return to air breathing, while those breathing fluorocarbon survive for weeks. The respiration of mice is optimally supported by these organic liquids at about 20°C. In cats, arterial oxygenation is excellent, but there is some impairment of carbon dioxide elimination. All animals have suffered some pulmonary damage from breathing fluorocarbon liquids. Continued investigation of organic fluid respiration may lead to development of a safe method to support the respiration of man by liquids equilibrated with gases at atmospheric pressure.*

Oxygen is at least ten times as soluble in silicone and fluorochemical liquids as in plasma or saline. A given volume of oxygen-saturated silicone oil contains half-again as much oxygen as the same volume of air or whole blood, while a given volume of oxygen-saturated fluorochemical liquid contains three times as much (*1*, *2*). It therefore seems reasonable to expect that they might be capable of supporting the respiration of intact animals. These organic liquids are available in viscosities near that of water, they are poor solvents, and are generally regarded as biologically inert (*3*). The silicone oils (polymethylsiloxanes) are immiscible with the fluorocarbon liquids (perfluorobutyltetrahydrofurans) and with water. Both have significant vapor pressures.

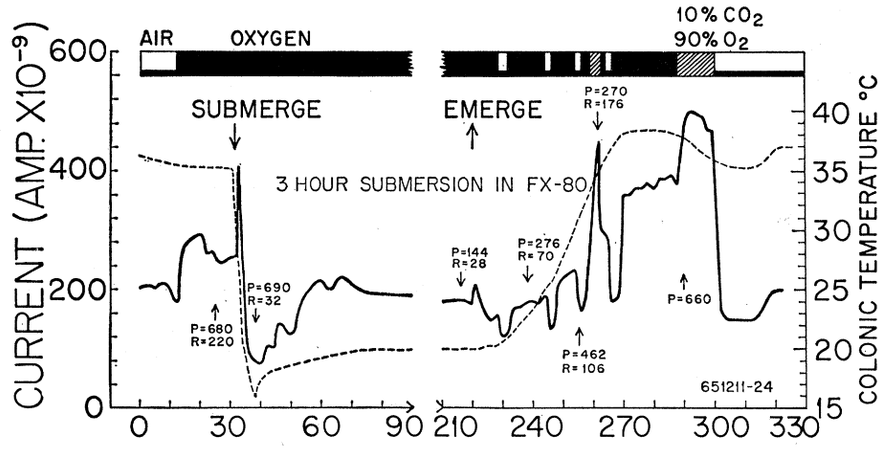

Fig. 1. Brain oxygen cathode current during liquid fluorocarbon breathing in the mouse. The polarographic brain oxygen cathode current is indicated as the solid line and colonic temperature as the dotted line. Time in minutes is shown on the horizontal axis. Pulse and respiration rates are indicated by *P* and *R*. The gas being breathed or being equilibrated with the fluorocarbon is indicated on the bar at the top of the graph. Cathode: platinum, 40 gauge, 4 mm; reference: AgCl-Ag; respiration fluid: fluorocarbon FX-80, continuously bubbled with the gas indicated.

Preliminary experiments (*4*) indicated that mice and rats could survive complete immersion in oxygen-saturated silicone oils for prolonged periods and that the length of time they were able to continue breathing the oils was related to its viscosity and temperature. Thus, in groups of five mice, survival averaged 4, 5, 16, and 35 minutes in oxygen-saturated oil at 24°C, having viscosities of 10, 5, 2, and 1 centistoke, respectively. Silicone oils having a viscosity of 0.65 centistoke proved to be too toxic to use. The optimum temperature for survival was about 18°C; several animals breathed oil at this temperature for over 6 hours, while respiration usually ceased after 5 minutes at 35°C. The mean brain oxygen cathode current (*5*) at 20°C was approximately half that obtained during air breathing at the same temperature. Several cats survived respiration with 1-centistoke silicone oil for 1 hour, but the arterial pO_2 fell, the pCO_2 increased, and the *p*H decreased. All of the mammals succumbed between 10 minutes and 5 hours after removal from the silicone fluid. Goldfish survived under silicone oil for several weeks.

Mice breathing the liquid fluorocarbon for 1 hour, in contrast to those breathing the silicone oils, survived for several weeks after removal from the fluid. Immersion survival times averaged about 4 hours at 18°C, 40 minutes at 25°C, and 15 minutes at 30°C. One animal continued to breath the liquid for 20 hours at 18°. Schlieren could be seen in the expired liquid even after the temperatures of the mice were the same as that of the oil, indicating a change in the refractive index or density of the liquid, depending on its gas content. The addition of Fluothane (1 cm^3 per liter of FX-80) arrests the swimming motions of the mice when submerged and the animals survive.

Brain oxygen cathode currents were recorded in a number of mice before, during, and after immersion in the fluorocarbon and a typical result is shown in Fig. 1. It can be seen that the cerebral oxygen tension during fluid breathing is roughly equivalent to that

observed during air breathing and that after the animal is removed from the liquid, the brain oxygen level returns to control values. If the liquid temperature is increased from 20° to 35°C while the animal remains submerged, the brain oxygen current rapidly falls to zero. If the pO_2 of the liquid is lowered from 600 to 140 while the animal remains submerged at 20°C, the brain oxygen cathode current decreases to zero.

Anesthetized cats spontaneously respiring oxygen-bubbled fluorocarbon through a tracheal cannula maintain arterial oxygen tensions between 140 and 300 mm, using tidal volumes between 12 and 60 cm^3 and endotracheal pressures of 10 to 15 cm of water. The arterial pCO_2 increases from 50 to 80 mm and the pH falls from 7.35 to 7.10. On return of the cats to O_2 breathing, the arterial pO_2 ranges between 300 and 400, the pCO_2 between 40 and 44, and the pH between 7.33 and 7.39. Arterial blood pressure ranges between 70 and 135 mm. One cat was observed for 5 days following liquid breathing; this animal walked about and drank milk but was in respiratory distress during this time and succumbed within 15 minutes after the subcutaneous administration of hydrocortisone (50 mg), with copious loss of bloody fluid from the trachea. All of the organs were grossly normal except the lungs, which appeared congested when collapsed but appeared normal when inflated. Several of the apparently normal mice sacrificed on the fifth day showed red areas distributed on the lungs in a polka-dot pattern.

A mouse supported in a stoppered, inverted funnel held so that the large end is just below the surface of a slowly stirred liquid, and having a gas volume of 125 cm^3 and a gas-liquid interface of 50 cm^2, survives for hours if air-saturated fluorocarbon is used as the liquid but succumbs within 20 minutes if air-saturated water is used.

The diffusion of oxygen through the fluorocarbon is four times as fast as through saline, as measured by an oxygen electrode and liquid-soaked filter paper.

Mice survive the breathing of oxygen saturated with fluorocarbon vapor for over 24 hours, and survive as well the intraperitoneal, subcutaneous, and even the intravenous injection of 2 cm^3 of FX-80. Silicone oils and fluorocarbon appeared to improve, with use, in their ability to maintain life during fluid respiration.

The observations in mice strongly suggest that the tracheal diameter limits the gas exchange, meeting the requirements only in hypothermia, at a high fluid pO_2, and a viscosity near that of water. In the cat, arterial oxygenation is entirely adequate but carbon dioxide elimination is impaired.

These findings resemble those of Kylstra (*6*), who studied the respiration of saline and other liquids hyperbarically equilibrated with oxygen and concluded that pulmonary gas exchange in liquid-ventilated lungs is diffusion limited. Fluorochemical liquid respiration should prove to be more efficient than aqueous liquid respiration because of its remarkably higher solubility for oxygen and carbon dioxide, its higher diffusion coefficient for gases, and its somewhat lower viscosity.

Whether the pulmonary damage observed is due to solvent activity, the presence of toxic impurities, a chemical interaction of the fluorocarbon structure with the lung, or some other factor is not yet clear. It is certain that the fluorocarbon liquid is superior to the silicone oils.

These organic liquids should prove to be of value in studies of gas exchange in living tissues and animals. Organic liquids, since they can support respiration with oxygen at atmospheric pressure and have other unique qualities, may find use in submarine escape, undersea oxygen support facilities, and medical research. The pulmonary damage caused by the breathing of the organic liquids available at the present time remains a major complication of their use in man.

Note added in proof. Since this report was submitted for publication, Kylstra *et al.* [*J. Appl. Physiol.* **21**, 177 (1966)] have reported survival of dogs ventilated with hyperbarically oxygenated, modified Ringer solution.

LELAND C. CLARK, JR.
Department of Surgery, Medical College of Alabama, Birmingham
FRANK GOLLAN
Radioisotope Service, Veterans Administration Hospital, University of Miami School of Medicine, Coral Gables, Florida

References and Notes

1. R. Rodnight, *Biochem J.* **57**, 661 (1954), Silas Braley, Dow Corning Co., Midland, Mich., reports oxygen solubility as 1.75 ml per milliliter of D. C. 200 fluid, 50 centistoke (personal communication).
2. Solubility of oxygen in FC-75, a commercially available form of FX-80, is reported by the 3M Co., St. Paul, Minnesota, as 65 percent, by volume, at room temperature. Our preliminary measurements confirm this value.
3. V. K. Rowe, H. C. Spencer, S. L. Bass, *J. Ind. Hyg. Toxicol.* **30**, 332 (1948); D. Gloxhuber and G. Hecht, *Arzneimittel-Forsch.* **5**, 10 (1955); S. Howlett, D. Dundas, D. C. Sabiston, *Arch. Surg.* **91**, 643 (1965); Minnesota Mining and Manufacturing Co., Chemical Div., Technical Information, *3M Brand Inert Fluorochemical Liquids* (1965).
4. L. C. Clark, Jr., unpublished observations.
5. ———, G. Misrahy, R. P. Fox, *J. Appl. Physiol.* **13**, 85 (1958).
6. J. A. Kylstra, M. O. Tissing, A. van der Maen, *Trans. Am. Soc. Artificial Internal Organs* **8**, 378 (1962).
7. Supported in part by NIH grants HE-06353, HE-03109, and a Career Award to L.C.C. We are indebted to Silas Braley, Dow Corning Co., Midland, Mich., to the Union Carbide Co., and to Lou Cove, 3M Company, St. Paul, Minn., for providing samples of fluids.

3 January 1966; revised 4 April 1966. ■

Actin: Volume Change on Transformation of G-Form to F-Form

Abstract. *The volume change occurring on polymerizing actin was measured by dilatometry. A large positive value of + 391 ml/mole was obtained for the volume change during the transformation of G- to F-actin. This large increase in volume could be interpreted as arising from the local change in the ordered water structure on the protein's surface at polymerizing sites.*

The muscle protein, actin, characteristically exists as a globular monomer, G-actin, in salt-free solutions, and it is polymerized by the addition of salt (*1*) to a fibrous double-stranded helical molecule, F-actin (*2*). A study (*3*) of the effect of pressure on actin solutions showed that F-actin may be easily depolymerized in the presence of adenosine triphosphate (ATP) by applying a pressure of approximately 2500 kg/cm^2. This result suggests that actin in the G-form has a smaller volume than in the F-form, since Le Chatelier's principle may be applied to this system. Furthermore, the G–F transformation is favored by elevating temperature, that is, the polymerization is an endothermic reaction (*4*). Recently, Hayashi *et al.* (*5*) have obtained a highly purified G–ADP (adenosine diphosphate) actin which shows the reversible G–F transformation simply by varying the temperature. Analysis of their results indicates that this transformation is endothermic, with large