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

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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

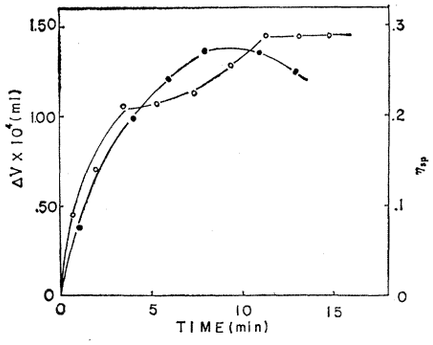

Fig. 1. Time course of the volume and viscosity increases of ATP–G-actin solution on polymerization. At zero time, 15 ml of G-actin solution (1.15 mg/ml) in 2 m*M* tris-HCl buffer (*p*H 8.0) containing 500 μM ATP was mixed with 3 ml of 6 m*M* $MgCl_2$. Temperature was maintained at 25° ± 0.0002°C. ○, Volume change; ●, viscosity.

positive values of molar enthalpy and entropy. The large, positive entropy change accompanying the G–F transformation is unlikely to be due solely to conformational change occurring during the transformation, but may arise from a melting of the ordered water structure on the protein surface (*6*). This result also suggests that a large volume increase is expected when actin becomes polymerized.

To verify the aforementioned expectation, the volume change on polymerizing G- to F-actin at 25°C was measured directly with Linderstrøm-Lang type dilatometers (*1*). For the purpose of obtaining the molar volume of a G-actin unit associated with the G–F transformation, corrections should be made for additional volume changes accompanying the mixing of the protein solution and the polymerizing solvent. The largest correction comes from the volume increase due to the binding of ATP and Mg ions, which has been previously reported to be 22 ml/mole (*8*); this value was confirmed by our measurements. The volume increase due to such binding can be estimated by assuming that all the ATP molecules in the G-actin solution bind to Mg ions in the polymerizing solvent. Another effect, the change in volume on dilution of $MgCl_2$ or protein by mixing, was negligibly small. The volume change (ΔV) of the solution, after correction of the volume increase due to the formation of the Mg-ATP complex, increases as polymerization progresses. For this experiment, 3 ml of 0.006*M* $MgCl_2$ was added to 15 ml of a solution of G-actin (1.15 mg/ml in 2 m*M* tris-HCl buffer, *p*H 8.1) containing ATP (500 μM). At the earlier phases of the polymerization, the time course for the volume change is almost parallel with the increase in viscosity, which was measured by an Ostwald-type viscometer. After 12 minutes, the volume change reached a finite value, whereas the viscosity reached maximum at about 8 minutes. The occurrence of a peak in the course of the viscosity measurement (the "overshoot" observed) may be due to a small amount of contaminating α-actinin (*9*).

The final value for the volume increase after complete polymerization increases in direct proportion with the F-actin concentration (Fig. 2). From the slope of this line, an apparent volume change per monomer unit (having molecular weight of 57,000) is calculated to be 377 ml/mole. To this volume change, another correction should be made for the inorganic phosphate ions produced when the ATP associated with G-actin is dephosphorylated during the polymerization of G- to F-actin. According to Noguchi *et al.* (*7*), a volume decrease of 18.6 ml/mole of inorganic phosphate was obtained when one mole of ATP was hydrolyzed in a medium containing 0.06*M* KCl and tris-malate buffer (40 m*M*, *p*H 7.0). Therefore, a correction for the volume decrease due to production of inorganic phosphate can be made by measuring the amounts of inorganic phosphate in solutions before and after polymerization. The corrected values of the volume change on polymerizing G-actin was plotted against the protein concentration in Fig. 2. The volume increase as estimated from the slope is 391 ml per mole of protein. By comparison with molar volume changes for other phenomena (*10*), the volume change on the transformation of G- to F- actin is characteristic in its large positive value.

To minimize the need for corrections of the observed volume change due to nonprotein volume changes, it is desirable to do the same experiment on G–ADP-actin, which does not split nucleotide during the polymerization. A preliminary result showed almost the same (but a little smaller) value for the volume increase on polymerizing G–ADP-actin, further confirmation that a large increase in the volume of protein occurs on the transformation of G- to F-actin.

These results are in the direction expected and consistent with the assumption that polymerization results in

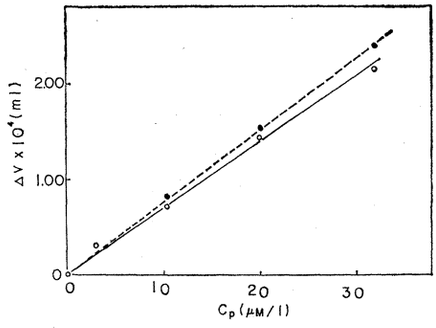

Fig. 2. Concentration dependence of the final volume change accompanying the transformation of G- to F-actin. ○, Volume change, ΔV, without correction for the liberation of inorganic phosphate ΔPi; ●, correction was made for the decrease in volume by ΔPi, C_p.

the melting of ordered water molecules on the protein's surface to some extent. Such a large volume increase during polymerization of actin could arise from several factors, such as conformational change of the protein, charge neutralization, or formation of hydrophobic bonds. The second- and third-mentioned factors would also contribute to the large entropy increase found on polymerization of G–ADP-actin (*5*), provided that the entropy change is attributed to the destruction of ordered water structure surrounding the protein. Further studies on the volume change thought to be helpful for elucidating the polymerization mechanism and nature of the bond between monomer units in F-actin are now in progress.

TAKAMITSU IKKAI
TATSUO OOI
Department of Physics,
Faculty of Science,
Nagoya University, Nagoya, Japan
HAJIME NOGUCHI
Aichi College of Liberal Arts,
Myodaiji-cho, Okazaki, Aichi, Japan

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