The Use of Fluorocarbon Emulsion in the Ussing Chamber[1] (34862)

G. SACHS, L. C. CLARK, AND G. M. MAKHLOUF

University of Alabama Medical Center, Birmingham, Alabama 35233; and University of Cincinnati, Cincinnati, Ohio 45221

The study of the biochemical processes related to H secretion has been seriously impeded by the low rates of secretion obtained *in vitro*. Davenport has shown that higher rates can be obtained if hyperbaric conditions are instituted (1). This suggests that inadequate oxygenation imposes usually a serious limitation on the secretory activity of parietal cells whose metabolism is largely mitochondrial and oxidative. This study shows that the problem of inadequate oxygenation can be partly overcome by the use of fluorocarbon, a liquid with high gas capacity (60 vol % O_2) (2).

Frog gastric mucosa stripped of external muscle layers, was mounted in an Ussing chamber, and acid rate, potential difference, resistance, and short circuit were measured by standard procedures (3). The circulation on the mucosal side was maintained by an airlift system utilizing 95% O_2 and 5% CO_2. The serosal side was circulated by a peristaltic pump, the outflow of which passed through a Teflon gassing coil immediately prior to entry into the chamber. Gassing on this side was also with 95% O_2 and 5% CO_2. The solutions used were: mucosal solution 100 m*M* Na, 5 m*M* K, 105 m*M* Cl with the pH stat set at 3.8; serosal solution standard frog Ringer emulsified with 20% (v/v) FC80 fluorocarbon (perfluoro tributylamine) using pluronic F68 (Wyandotte Chemical Corporation) 8% as emulsifier. Emulsification was carried out by sonicating in ice for 1 hr with a Brownill sonic probe at maximum intensity. Inhibitors or stimulants of secretion were added directly to the serosal solution.

Secretory rates obtained from *Rana pipiens* gastric mucosa range from 1 to 5 μE cm^{-2} hr^{-1}, though occasionally higher rates are observed in the initial period of an experiment. In this, as in other laboratories, the mean secretory rate in the presence of histamine has been about 3.5 μE cm^{-2} hr^{-1}. The use of a peristaltic pump and Teflon oxygenator increased secretion but slightly in a given mucosa. Introduction of a fluorocarbon emulsion resulted in much higher secretory rates. Table I shows that the mean secretory rate in 15 experiments rose from 3.5 to 9.7 μeq cm^{-2} hr^{-1}. SCN^- or dinitrophenol abolished this acid rate, while diamox both under open and short-circuit conditions reduced it to control levels (Fig. 1). Gassing the emulsion with air increased secretion relative to air-gassed standard solutions, but did not give high acid rates as were obtained with 95% O_2, 5% CO_2 mixtures. A direct action of fluorocarbon can be ruled out by the above considerations, and also because these substances are extremely inert, being virtually immiscible with aqueous solutions. Accordingly, unless the suspension is adequately

[1] Supported by NIH TIAM Grant 2A-5286, N.S.F. Grant GB-8351.

TABLE I. The Effect of Fluorocarbon Emulsions on Acid Rate, Short Circuit Current, and Potential Difference of the Frog Gastric Mucosa.

	Acid (μeq cm^{-2} hr^{-1})	Short circuit current (μA cm^{-2} hr^{-1})	PD (mV)
Rana pipiens			
Control	3.8 ± 0.9	101.4 ± 5.7	22 ± 2
Fluorocarbon	9.6 ± 1.8	138.8 ± 11.6	27 ± 3.5

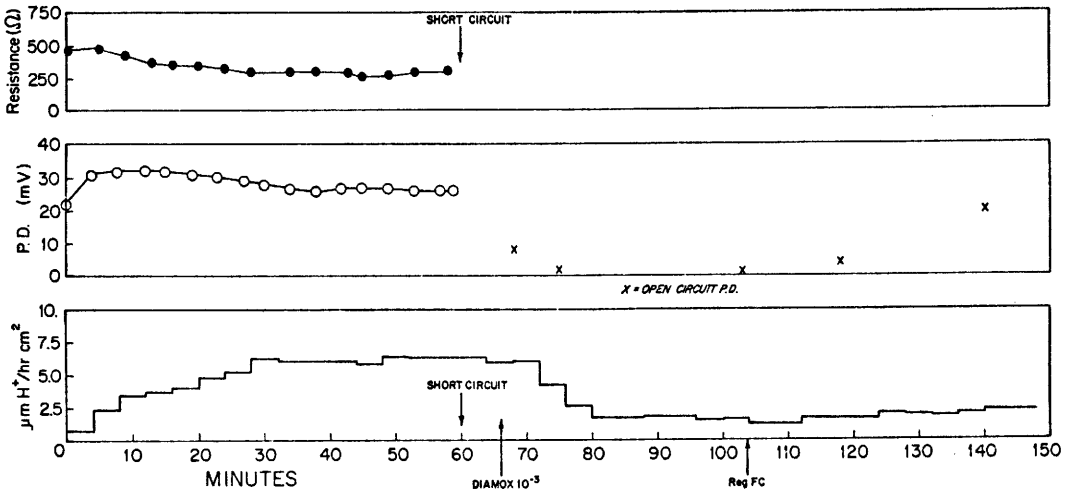

FIG. 1. The effect of 10^{-3} *M* diamox on acid secretion of the frog gastric mucosa using fluorocarbon emulsions as serosal bathing solutions. At time zero, fluorocarbon emulsion was substituted for standard solution on the serosal side. Diamox was added at 67 min and irreversibly reduced acid rate to control levels, as well as abolishing the PD. The effect on PD was reversible.

emulsified, no effect of fluorocarbon is discernible.

This study illustrates the potential value of fluorocarbon emulsions in eliciting increased activity from epithelial tissues *in vitro*. Although acid secretion increased threefold, it is probable that the limit of secretion was not attained since the distributive effect of the capillary circulation was absent. Combining fluorocarbon with some means of opening local circulatory channels may result in even higher rates than reported here.

The higher rates obtained permit a more confident comparison with *in vivo* preparations. The inhibitory effect of diamox deserves especial note. Carbonic anhydrase inhibitors reduce acid secretion *in vivo* (4), partly by permitting secreted acid to leak across a damaged surface epithelium. The residual secretion is interpreted as evidence for the dependence of acid secretion on a mechanism involving carbonic anhydrase either directly or through the maintenance of intracellular pH. In contrast, the low secretory rates obtained *in vitro* are resistant to diamox which exerts its effect on the Cl transport mechanism and its associated PD (5). The findings of this study shed some light on the apparent disparity. It is possible that low *in vitro* rates represent secretion supported by uncatalyzed rates of CO_2 hydration and thus resistant to the effect of diamox. Reversion of higher rates obtained by fluorocarbon to control levels on addition of diamox appears to support this suggestion.

1. Davenport, H. W., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **11**, 715 (1952).

2. Clark, L. C., and Gollan, F., Science **152**, 1755 (1966).

3. Heinz, E., and Durbin, R. P., Biochem. Biophys. Acta **31**, 246 (1959).

4. Cain, S. M., Stainsby, W. N., and Otis, A. B., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **18**, 22 (1959).

5. Hogben, C. A. M., "Electrolytes in Biological Systems," p. 176. Amer. Physiol. Soc., Washington, D.C. (1955).

Received Mar. 16, 1970. P.S.E.B.M., 1970, Vol. 134.