

## Oxygen consumption of the frog gastric mucosa

The gastric mucosa of the frog *in vitro* maintains an electrical potential difference and secretes HCl for many hours. Both electrical and secretory activity are strongly dependent on respiration, disappearing quickly after removal of external oxygen<sup>1</sup>. The molar ratio of HCl secreted to O<sub>2</sub> consumed by the mucosa has been used to evaluate various proposed mechanisms for the production of acid<sup>2</sup>. Previous estimates of the ratio<sup>3,4</sup> have been based on the manometric measurement of O<sub>2</sub> consumption in the absence of external CO<sub>2</sub>, although the latter gas is necessary for maximal acid secretion<sup>5</sup>. This method also precluded the detection of changes in electrical activity and any associated changes in O<sub>2</sub> consumption.

These experimental difficulties may be eliminated by the use of the polarographic method for the measurement of the O<sub>2</sub> concentration in the solutions bathing the isolated mucosa. In our experiments the mucosal membrane was mounted between two Lucite chambers, one containing a modified Krebs solution in contact with the serosal surface and the other chamber containing a similar solution, without bicarbonate buffer, in contact with the mucosal surface. These solutions had been equilibrated with 95 % O<sub>2</sub> + 5 % CO<sub>2</sub> and pure O<sub>2</sub> respectively. The concentration of O<sub>2</sub> in each solution was measured by an O<sub>2</sub> electrode<sup>6</sup> and always exceeded 50 % saturation. An electrical circuit permitted the continuous measurement of the current delivered by the mucosa into an external resistance of zero, *i.e.*, the short-circuit current. Acid secretion was measured by electrometric titration *in situ*. The solutions were stirred magnetically and were closed to the atmosphere. Measurements of the rates of acid secretion and O<sub>2</sub> removal from the two solutions were made for two to four successive intervals of 50 to 60 min each.

It was found that the rates of removal of O<sub>2</sub> from the two solutions were comparable; these two values have been added to find the total rate of O<sub>2</sub> consumption. All results have been expressed in  $\mu$ moles or  $\mu$ equiv./mg dry wt. of mucosa/h. Table I lists the means, with corresponding standard errors, for 42 periods during which the mucosae were secreting spontaneously, and for 15 periods during which the mucosae were stimulated by  $5 \cdot 10^{-5}$  M histamine.

TABLE I

	Acid secretion ( $\mu$ equiv./mg/h)	Short-circuit current ( $\mu$ equiv./mg/h)	O <sub>2</sub> consumption ( $\mu$ moles/mg/h)
Spontaneous (42 periods)	0.079	0.362	0.139
S.E. *	$\pm 0.010$	$\pm 0.023$	$\pm 0.011$
Stimulated (15 periods)	0.150	0.360	0.168
S.E. *	$\pm 0.021$	$\pm 0.027$	$\pm 0.015$

\* Standard error.

As found previously<sup>7</sup>, the short-circuit current in these experiments does not depend on the rate of acid secretion. This finding makes possible an estimate of the ratio  $\Delta H/\Delta O_2$  from Table I. Using the means of acid secretion and O<sub>2</sub> consumption,  $\Delta H/\Delta O_2 = 0.071/0.029 = 2.4$ .

In an alternate analysis, it was assumed that the data could be fitted to an equation of the form:

$$\begin{aligned}\text{Oxygen consumption} &= A + k_1 (\text{acid secretion}) \\ &\quad + k_2 (\text{short-circuit current})\end{aligned}$$

The constant  $A$  represents the  $O_2$  consumed in processes other than those responsible for current or acid production. The values for  $k_1$ ,  $k_2$  and  $A$  were obtained by a conventional, least-squares technique<sup>8</sup>. A preliminary calculation showed that the use of histamine did not significantly affect the value of  $A$ , and the 57 experimental periods were combined to yield the values:

$$\begin{aligned}k_1 & 0.41 \pm 0.08 \text{ (standard deviation)} \\ k_2 & 0.27 \pm 0.04 \\ A & 0.01 \pm 0.05\end{aligned}$$

By the use of the  $t$  test, in which  $t_1 = 0.41/0.08 = 5.1$  and  $t = 0.27/0.04 = 6.7$ , we may virtually exclude the possibility that the  $O_2$  consumption is independent of acid secretion ( $k_1 = 0$ ) or short-circuit current ( $k_2 = 0$ ). The value obtained for  $A$  indicates that nearly all the respiration is related to secretory and electrical activity.

The reciprocal of  $k_1$ ,  $1/0.41 = 2.4$ , gives  $\Delta H/\Delta O_2$  at constant short-circuit current; the reciprocal of  $k_2$ ,  $1/0.27 = 3.7$ , yields  $\Delta$  (short-circuit current)/ $\Delta O_2$  at constant acid secretion. CRANE AND DAVIES<sup>3</sup> found for the ratio  $\Delta H/\Delta O_2$  values up to 13, with only one-third of the measurements yielding a value less than 4. DAVENPORT<sup>4</sup> has, however, reported a value of 2.15 for this ratio. The present results (and those of DAVENPORT) are consistent with the hypothesis that secreted  $H^+$  is derived by a "redox" mechanism from substrate hydrogen (*cf.* ref. 9). A similar mechanism, presumed separate<sup>10</sup>, may give rise to the transport of  $Cl^-$  manifested by the electrical current. These results leave open the question of linkage between the two mechanisms.

This work was supported by grants from the American Heart Association and the National Science Foundation (NSF - G 9795).

Biophysical Laboratory Harvard Medical School,  
Boston, Mass. (U.S.A.)

LEOPOLDO VILLEGAS\*  
RICHARD DURBIN\*\*

<sup>1</sup> E. E. CRANE, R. E. DAVIES AND N. M. LONGMUIR, *Biochem. J.*, 43 (1948) 321.

<sup>2</sup> E. HEINZ AND K. J. ÖBRINK, *Physiol. Rev.*, 34 (1954) 643.

<sup>3</sup> E. E. CRANE AND R. E. DAVIES, *Biochem. J.*, 49 (1951) 169.

<sup>4</sup> H. W. DAVENPORT, *Fed. Proc.*, 11 (1952) 715.

<sup>5</sup> C. A. M. HOGBEN, in A. M. SHANES, *Electrolytes in Biological Systems*, American Physiological Society, Washington, 1955.

<sup>6</sup> L. C. CLARK, JR., *Trans. Am. Soc. for Int. Organs*, 11 (1956) 41.

<sup>7</sup> R. P. DURBIN AND E. HEINZ, *J. Gen. Physiol.*, 41 (1958) 1035.

<sup>8</sup> G. W. SNEDECOR, *Statistical Methods*, Iowa State College Press, Ames, 1957.

<sup>9</sup> E. J. CONWAY, in *The Method of Isotopic Tracers Applied to the Study of Active Ion Transport* (Saclay), Pergamon Press, New York, 1959.

<sup>10</sup> E. HEINZ AND R. P. DURBIN, *Biochim. Biophys. Acta*, 31 (1959) 246.

\* On leave of absence from the Biophysical Laboratory, Instituto Venezolano de Investigaciones Científicas, Caracas.

\*\* Established Investigator of the American Heart Association.

Received August 26th, 1960

*Biochim. Biophys. Acta*, 44 (1960) 612-613