CYTOCHROMES AND ACID SECRETION IN THE GASTRIC MUCOSA OF RAJA ERINACEA

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Acid secretion in the gastric mucosa of many species is completely dependent upon oxygen availability. In the frog gastric mucosa, the cytochromes (and particularly cytochrome c) are 25 to 75% reduced in the secreting steady state achieved in the presence of 0.9 atm O_2, and become completely reduced in the absence of O_2. Upon reoxygenation, cytochrome c becomes fully oxidized, and only returns to its reduced steady state as acid secretion resumes following a lag of several minutes. (Kidder et al., Am. J. Physiol. 211:513, 1966) This behaviour supports an hypothesis in which gastric acid secretion derives its energy from the cytochrome system.

The elasmobranch gastric mucosa is similar to that of the frog in many respects, but requires oxygen tensions above 1 atm for maximal secretory rate. (Kidder, Am. J. Physiol. 231:1240, 1976) The spectrophotometer is a modification of the multi-beam spectrophotometer previously described (Kidder & Blankemeyer, J. Biol. Physics 5:119, 1978), which can now produce scanning spectra as well as dual-wavelength monitoring of a tissue under hyperbaric conditions. Gastric mucosa from the little skate (Raja erinacea) was dissected and mounted in an Ussing chamber, using methods and solutions previously employed (Kidder & Kidder, Bull. MDIBL 24:110, 1984). Hyperbaric pressure was maintained at 2 atm, using 90.25% O_2 + 4.75% CO_2 + 5% CO as the gas phase.

Figure 1 shows a portion of a typical experiment. Starting in the secreting steady state after equilibration with the hyperbaric conditions, three cytochromes are monitored along with the transepithelial potential difference (PD) and the acid secretory rate. Replacing O_2 with N_2 at 20 minutes completely inhibits acid secretion and results in a reduction of the cytochromes, presumably to a fully reduced state. Returning to O_2 at 40 min. reoxidizes the cytochromes, but to a level more oxidized than the steady

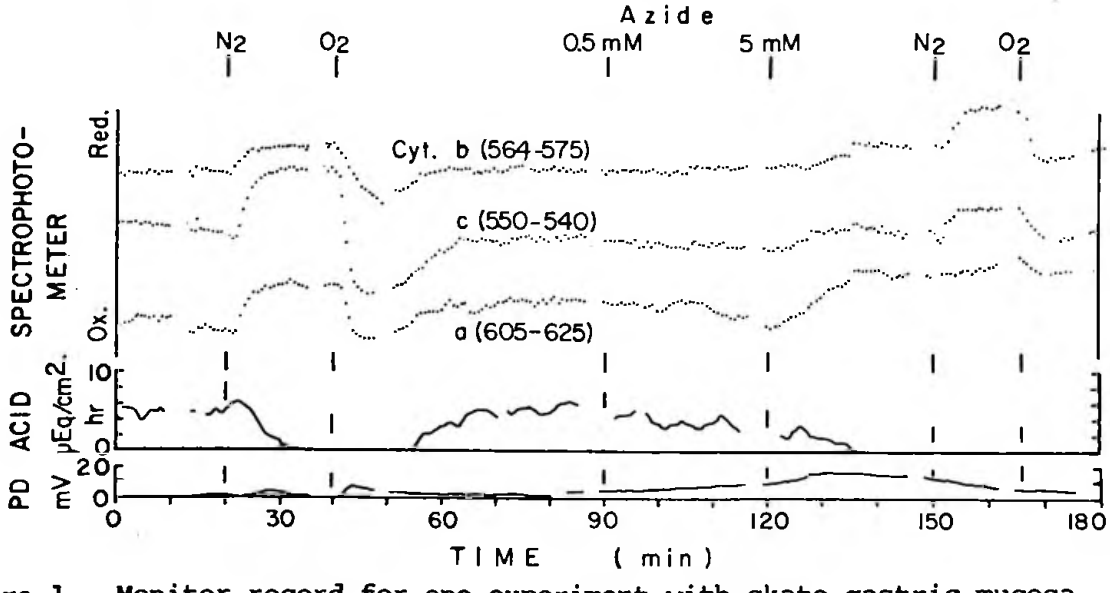

Figure 1. Monitor record for one experiment with skate gastric mucosa.

state. As acid secretion resumes (50 minutes), the cytochromes are reduced to levels approximating the previous steady state. This behaviour is similar to that observed in the frog mucosa.

Scanning spectra were obtained at significant points during these changes. The reference spectrum is the peak of the oxidized "overshoot" following reoxygenation; this is the most oxidized state obtainable in frog. Figure 2 shows the difference spectrum N_2 minus oxidized, which is a typical cytochrome spectrum with peaks at 550 (cyto. c) and 605 (cyto. $a+a_3$), a shoulder at 564 due to b cytochromes, and a large trough at 465 from flavoproteins. The peak at 605 is asymetrical due to the presence of some cytochrome a_3-CO complex, which has a peak at 590. Figure 2 also shows the corresponding difference spectrum between the secreting steady state and the fully oxidized state. The cytochromes are about 50% reduced in the steady state, with somewhat less reduction in $a+a_3$ and considerably less reduction in flavoprotein. The agreement between the scanning spectra and the dual-wavelength results gives confidence in the validity of both techniques.

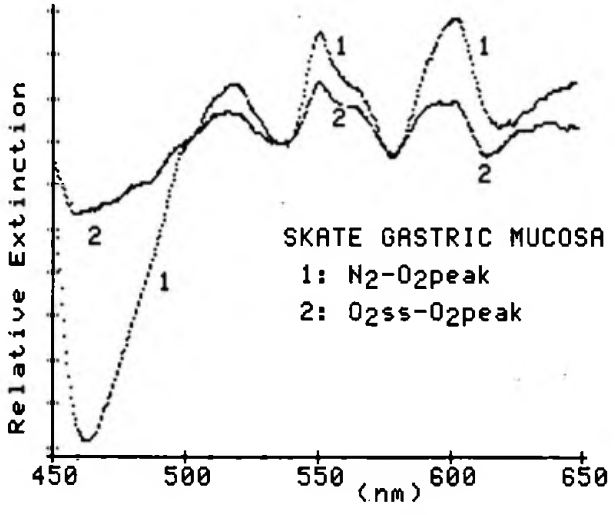

Figure 2 N_2 and steady state spectra

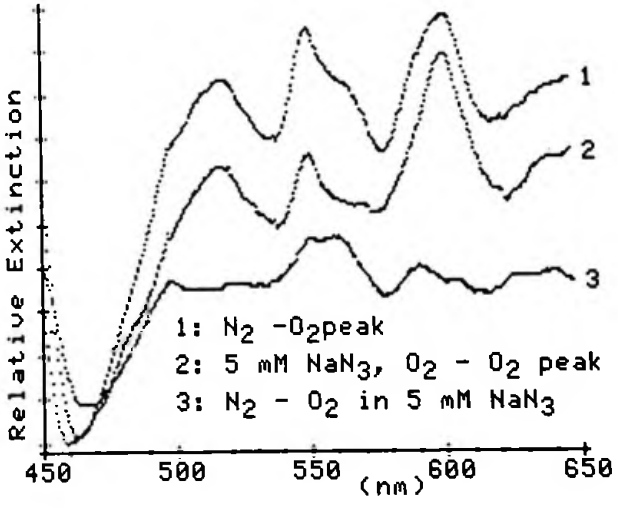

Figure 3 Azide spectra

Gastric acid secretion requires O_2, and should be sensitive to terminal oxidase inhibitors. The skate mucosa is rather insensitive to NaN_3, as seen by the response at 0.5 mM. 5 mM azide abolishes secretion, raises the PD, and reduces all 3 cytochromes. However, the response of cytochromes b and c to the subsequent removal of O_2 shows that O_2 still has an effect. Figure 3 shows the spectra. Although cytochrome oxidase is completely reduced by 5 mM azide, only part of the b, c and flavoprotein components have been reduced; the remainder are reduced by removal of O_2, which requires some alternate route of interaction between oxygen and the cytochrome system. It will be of interest to see whether the skate mucosa can secrete acid in high CO as the frog does (Kidder, Am. J. Physiol. 238:G197, 1980), and what CO does to the cytochrome system.

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