AZIDE INSENSITIVITY OF OXYGEN CONSUMPTION AND SOME CYTOCHROMES OF THE GASTRIC MUCOSA OF RAJA ERINACEA

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Acid secretion by the gastric mucosa of the little skate is a strictly aerobic process. Since this tissue contains an apparently normal cytochrome system (Kidder & Kidder, Bull. MDIBL 25:40, 1985) one would presume that the elimination of cytochrome oxidase activity by inhibitors should mimic the effect of anoxia. However, acid secretion is not abolished by a high CO/O_2 ratio, and much of the cytochrome system continues to react with O_2 under these conditions (Kidder & Kidder, Bull. MDIBL 26:43, 1987). Another potent cytochrome oxidase inhibitor is azide (N_3^-), which inhibits acid secretion in the frog gastric mucosa. However, there are differences between N_3^--inhibited and anoxic tissues which suggest that N_3^- is having additional effects on this tissue (Kidder, Am. J. Physiol. 246:G40, 1984). It was therefore of interest to determine the effects of this inhibitor on respiration and cytochromes in skate.

O_2 consumption was measured in a stainless steel Ussing-type chamber equipped with O_2 electrodes in each chamber half. After equilibrating the solutions (Forster's) with 90% O_2/10% CO_2, the chambers were isolated and the decreasing O_2 concentration was recorded. Since N_3^- effects are irreversible in this system, azide concentrations were never reduced. Rates in N_3^- are expressed as percentage of the control rate for that tissue.

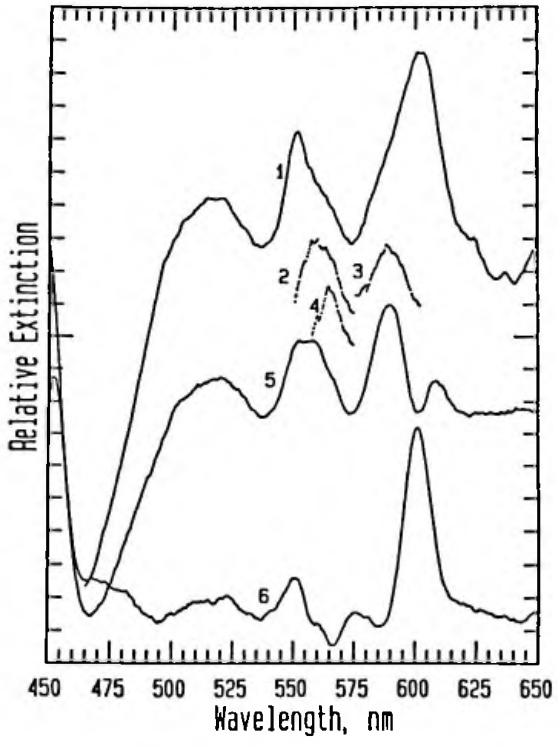
TABLE I
AZIDE AND RESPIRATION OF SKATE GASTRIC MUCOSA
(% of control)

[AZ] (mM)	SEROSAL Mean	±SE	MUCOSAL Mean	±SE	N
0.1	80.42	± 3.102	98.26	± 3.238	5
0.2	63.90	± 2.681	77.80	± 3.962	5
0.5	61.08	± 3.227	79.52	± 4.638	6
1.0	75.83	± 3.501	86.93	± 1.228	7
2.0	73.12	± 4.940	64.10	± 5.958	4
5.0	56.10	± 1.445	76.16	± 2.273	7
10.0	58.59	± 2.517	54.95	± 2.630	8
15.0	38.14	± 2.964	53.66	± 5.266	5
20.0	34.55	± 1.012	45.35	± 3.013	6
25.0	45.17	± 1.515	52.09	± 3.140	7
50.0	25.24	± 1.591	36.76	± 2.463	5
100.0	27.56	± 3.621	33.60	± 2.729	5

Table I shows these results for 11 tissues tested. All values are significantly different both from 100% and from 0%, with P less than 0.01 except for the serosal value at 2 mM, where P is below 0.05 for the difference from 100% due to the lower N. Note that while N_3^- inhibits 40% to 50% of respiration at low concentrations, the remainder is very insensitive to inhibition at concentrations up to 100 times the expected K_I for cytochrome oxidase.

When a Dixon plot is constructed for the respiration at N_3^- concentrations of 5 mM and above, the K_I's are estimated as 60.3 and 96.8 mM for the serosal and mucosal sides respectively (least squares fit to data, r = 0.60 and r = 0.46, N = 44 for each side).

Spectrophotometric studies employed the apparatus previously used. The control acid secretory rate was 2.25 $\pm$ 0.30 uEq/cm^2·hr (N = 15), dropping to 0.26 $\pm$ 0.11 uEq/cm^2·hr (7) in 1 mM N_3^- and to values not different from control at 5 and 25 mM.



Spectrum #1 is a N_2-O_2 difference spectrum in the absence of N_3^-, showing the trough near 465 nm (flavoprotein) and peaks near 550 (cyto. c) and 602 (cyto. a). Both of the cytochrome peaks are asymmetrical, and can be analyzed by assuming that they consist of at least two symmetrical components with different peak wavelengths and intensities. If the steeper side is due to the major component alone, we can reconstruct that major component by reflecting its steep side around the peak wavelength; when this reconstructed peak is subtracted from the observed spectrum, the remainder should be the minor peak(s). This deconvolution can be repeated if the minor peak is still asymmetrical.

Spectrum #2 is the result of subtracting the reconstructed 550 nm cytochrome c peak from the recorded spectrum, while spectrum #3 is the subtraction of the 602.5 nm cytochrome a peak. Spectrum #2 peaks at 557 nm, and is still asymmetrical. Subtraction of a symmetrical 557 peak from spectrum #2 yields spectrum #4, which peaks at 564 and is presumably cytochrome b, which is expected to be present. Spectrum #3 is a symmetrical peak at 590 nm, which will be seen below.

Spectrum #5 shows the N_2 minus O_2 difference spectrum in the presence of 5 mM N_3^-. Note first that most of the cytochrome b and most of the 557 component, and about half of the cytochrome c, are reacting with O_2. The cytochrome a peak is virtually abolished, unmasking the peak at 590 observed in spectrum #3. Finally, spectrum #6 is the difference between the N_3^--inhibited tissue in O_2 and the control tissue in O_2, and therefore shows those components reduced by N_3^-. A prominent 602.5 nm cytochrome oxidase peak appears; since this component is reduced by N_3^- in O_2, this accounts for its removal from the N_2 minus O_2 spectrum in the presence of N_3^-. A cytochrome c peak is also found in spectrum #6, accounting for the decrease in cytochrome c peak height in spectrum #5.

These findings have been repeated on 15 tissues for N_3^- concentrations of 1, 5 and 25 mM. The summary results agree with the conclusions stated above. By analogy to the peak positions of the known cytochromes, we speculate that the 590 peak might be an a-type cytochrome and function as an alternate cytochrome oxidase, with a high K_I for N_3^-. The 557 component likewise might be a c-type cytochrome involved in the acid secretory cytochrome chain which has been postulated to be responsible for providing the energy for gastric acid secretion (Kidder, Ann. N. Y. Acad. Sci. 341:259-273, 1980).

[Sup. by Ill. St. Univ. ORSP and URPP]