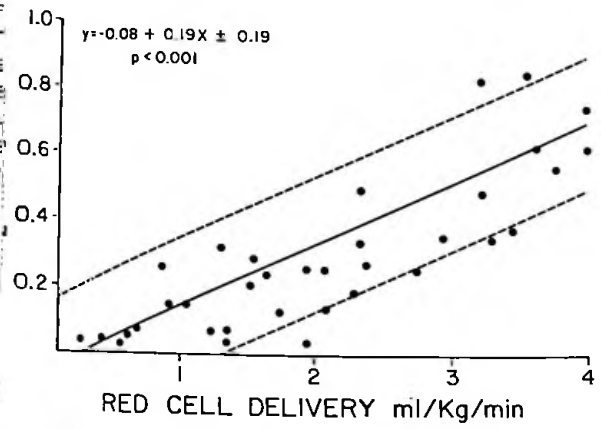
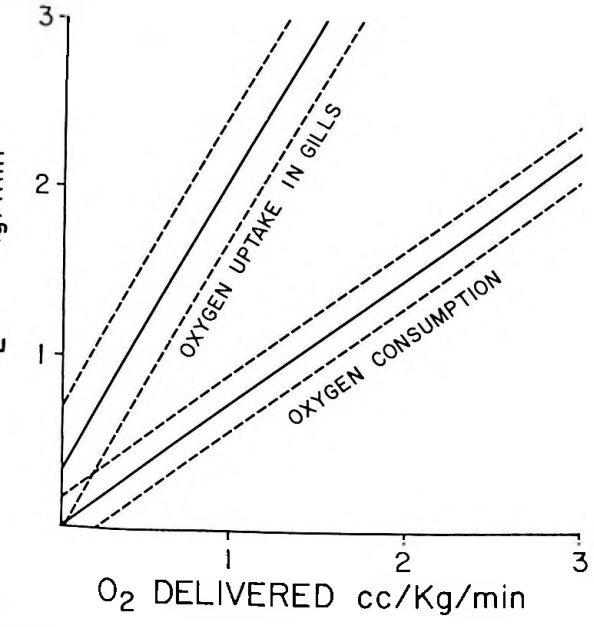
nute period. Cardiac output was recorded continuously nd arterial and venous blood samples for oxygen content etermination and hematocrit were taken at 15 minute tervals. VO_2 was calculated as $(caO_2\text{-}cvO_2) \times Q_B/100$.



gure 2. Cardiac output corrected for hematocrit vs. ygen consumption in 9 fish.

When thirty-six values of cardiac output from nine sh were compared with simultaneously measured ygen consumption no correlation was found (Figure 1). here was a significant correlation, however, between red ood flow rate (cardiac output corrected for hematocrit) nd VO_2 (Figure 2). Figure 3 shows the correlation etween oxygen delivered to the tissues and oxygen nsumption. The least squares regression equation erived from these data is $y = 0.05 + 0.69x + 0.13$ where



re 3. Oxygen delivered to the gills vs. oxygen uptake wn in steep curve; oxygen delivered to the tissues vs. gen consumption shown on lower curve. Least square ession lines calculated from 36 data point.

$y = VO_2$ (oxygen consumption), $x = caO_2 x Q_B$ and 0.13 is the standard error of the estimate (SEE). The oxygen uptake in the gills is shown on the same coordinate axis in Figure 3 and is described by the equation $y = 0.27 + 1.8x + 0.42$ where $y = VO_2$ (oxygen uptake in the gills, $x = cvO_2 x Q_B$ and 0.42 is SEE. Both correlations are significantly different from zero at p 0.001.

The high degree of correlation between oxygen consumption and oxygen delivery to the tissues shows the dogfish to be a true oxygen uptake conformer over a wide range of supplied oxygen. Oxygen consumption is independent of cardiac output which must be corrected for hematocrit in order to derive a meaningful relationship.

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Hyperbaric Experiments: A Test for Oxygen Sufficiency in *Squalus Acanthias* Gastric Mucosa in Vitro

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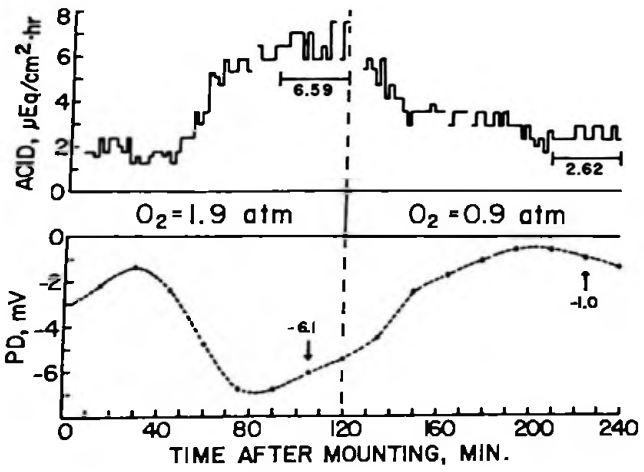
In the isolated chambered gastric mucosa of the dogfish, *Squalus acanthias*, diffusion of CO_2 into the tissue from the commonly-used 5% CO_2 supply is rate-limiting on the acid secretory rate. Use of 10% CO_2 gives a significant rise in rate and allows the development of a small potential difference (PD). (*Kidder, Bull. MDIBL 14:58, 1974*). This effect is also seen in the bullfrog gastric mucosa (*Kidder and Montegomery, Am. J. Physiol. 227:300, 1974*). One can calculate that in the frog tissue, diffusion of O_2 into the tissue should be sufficient for its respiratory needs, and experiments confirm this conclusion [*Kidder and Montegomery, Am. J. Physiol. 229: in press*). For the thicker dogfish gastric mucosa, with its unknown repiratory rate, it seemed possible that the diffusion of oxygen into the tissue from its supply concentration of 0.9 atm (10% CO_2/ 90% O_2 at atmospheric pressure) might be rate-limiting, with the resulting hypoxia contributing to low secretory rates and PD. This hypothesis has been experimentally tested.

A standard chamber system was used to mount a segment of dogfish gastric mucosa (stripped of superficial muscle) between two fluid-filled chambers (3.14 cm^2 exposed surface.) The fluids were those of Hogben (*Science 129:1224, 1959*) and approximate the ionic composition of dogfish blood. Carbachol (2.5×10^{-4} M) was added to the serosal solution as a secretagogue. The inlet and outlet ports of the tissue chamber were arranged for efficient mixing; circulation and aeration were by "air lifts" in the fluid exit lines. Acid secretory rate was measured by the pH-state technique, and PD by KCL/calomel electrodes in the fluid lines. Resistance was measured by passing a 20 uA current pulse via Ag/AgCl electrodes remote from the tissue, measuring the resulting voltage change at 1 sec. The input gas, either

5% or 10% CO_2 in O_2, was hydrated in a bubbler and maintained at a constant 10 cmH_2O pressure difference between the gas line and the atmosphere around the tissue chamber by a simple "spillover" system to ensure a constant bubbling rate.

To raise pO_2 above 0.9 atm, the entire chamber system above was inserted into a small hyperbaric chamber system, which allowed application of 2 atm (15 psig) total pressure. Electrical leads for the pH, PD and current electrodes were passed through the pressure chamber wall in gas-tight syringe and valve assembly, using a polyethylene tube passed into the pressure system through another seal. When the total pressure was raised from 1 to 2 atm, the gas was changed from 10% CO_2 in O_2, which maintained pCO_2 at 0.1 atm as pO_2 changed from 0.9 to 1.9 atm.

Figure 1 shows the results of a typical experiment. The tissue was removed from the freshly-killed fish into ice-cold solution, dissected and mounted as rapidly as possible (15 min.), and stimulated with carbachol. Since hypoxia was suspected in lower oxygen conditions, the higher pressure was always applied first, to avoid hypoxic tissue damage. It can be seen that the acid secretory rate responds slowly to increased pO_2, reaching a level several-fold higher than the initial value. Changing to pO_2 of 0.9 atm slowly reverses this change. In many experiments, a third period of high O_2 and approaching zero in 0.9 atm O_2. (PD is measured reference mucosal = 0; this is the same convention used for the frog, in which the measured PD is serosal positive.) These changes are likewise slow, and seem to parallel the changes in secretory rate.



To enable comparisons in a series of tissues, average acid secretory rates were determined during the last 30 minutes of the 2 hour periods, and PD was recorded at the midpoint of these 30 min. periods. The averaged results for a series of tissues are shown in Fig. 2, along with similar averages from the previous summer's data. While increased pCO_2 gives a definite rise in secretory rate, and allows development of a significant PD, the increase in pO_2 causes much more dramatic changes. It is clear that the conditions represented by the first set of bars, which have been the conditions conventionally used for this and other mucosae, are suboptimal for this tissue.

Tissue resistance could not be measured in experiments in which the pH-stat was used, due to technical problems with electrical leakage in the hyperbaric chamber system. therefore, a separate series of 5 tissues were mounted between identical HCO_3-buffered solutions (usually used on the serosal side only) and subjected to the alternation of o_2 pressure as above, without using the pH-stat. The results are shown in Figure 3. Again, we find that PD rises in high O_2 and falls in low O_2. Resistance falls in high O_2 and rises in low O_2, consistent with the general observation in other gastric mucosae that conditions which increase the secretory rate reduce the resistance, and *vice versa*.

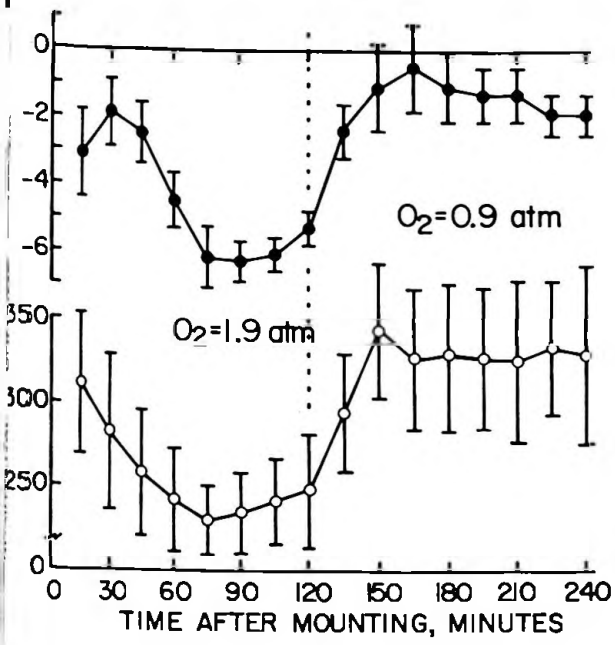
Two major points emerge from this study. First, it is clear that one cannot supply sufficient O_2 for maximum acid secretory rate in the *in vitro* preparation at one atmosphere total pressure. It is not known whether pO_2 = 1.9 atm is sufficient, although one experiment at a total pressure of 3 atm failed to give a further rise in acid secretory rate. In the intact animal, capillary circulation decreases the effective diffusion distance for gas exchange; thus oxygenation is probably sufficient *in vivo*. It should be remembered that the normal *in vivo* pressure on the mucosa is frequently well in excess of 2 atm (the fish are caught below 10 meters depth); this pressure is thus not "unphysiological".

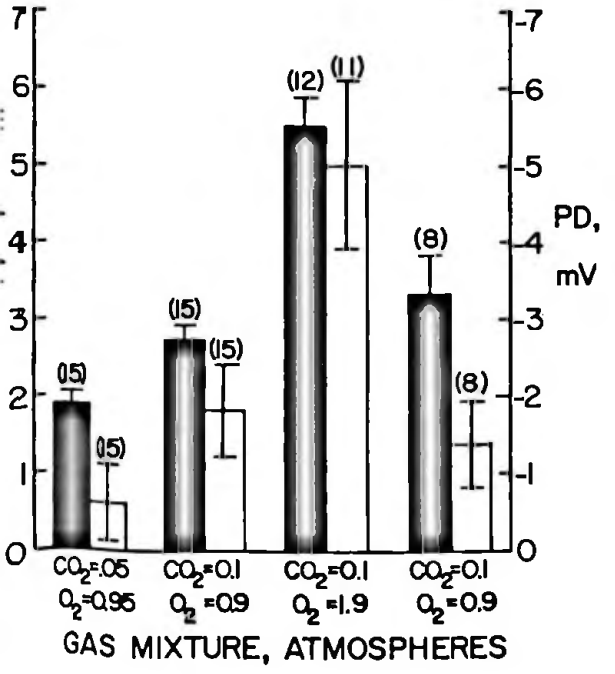
Secondly, the observation of a PD of 5 mV or so, oriented mucosal positive, has implications for the mechanism proposed for gastric acid secretion. In other gastric epithelia, the mucosal-negative PD found in most tissues is due to a somewhat higher activity of the Cl^- pump relative to the H^+ pump. If H^+ is transported by a neutral pump hypothesis), it is necessary to postulate an additional electrogenic Cl^- pump, oriented in the same direction, which is responsible for the PD. In the dogfish gastric mucosa, the existence of the mucosal-positive PD is explained by the electrogenic hypothesis by assuming that the H^+ pump is somewhat more active in this tissue than is the Cl^- pump. Stimulation of these pumps would account for the observed fall in resistance. In the neutral pump hypothesis, one would have to postulate that the electrogenic Cl^- pump is oriented to transport Cl^- from lumen to blood in order to account for the PD without introducing additional mechanisms. This seems unlikely, and taken at face value the observation of the mucosal-positive PD seems to support the electrogenic hypothesis.

Two cautions are necessary in reaching this conclusion. First, it is possible that the observed PD is due to the diffusion of HCl from the depths of the pits where it is secreted to the bulk mucosal solution. The higher mobility of H^+ relative to Cl^- would be expected to produce a few mV of PD, oriented mucosal positive. The available data do not permit rejection of this explanation for the observed PD. Secondly, this analysis is

sed on the assumption that in high O_2 the only ions ively transported are H^+ and Cl^-. This is known to be e for the low O_2 condition. If additional ions were nsported in high O_2, they could account for the ntation of the PD. Resolution of these problems must ait further experimentation.

Supported by N.S.F. GB-36278





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The Specificity of the Transport System for D-glactose at the Basal Face of Renal Tubular Cells of the Flounder (*Pseudopleuronectes americanus*)

Arnost Kleinzeller, George R. Dubyak and James M. Mullin. University of Pennsylvania School of Medicine, Philadelphia, Pa. 19174.

The structural specificity of the transport of D-galactose and 2-deoxy-D-galactose (0.5 mM) by teased tubules of the winter flounder was investigated. The uptake of sugars by the tubule reflects, preponderantly, transport events at the antiluminal (basal) membrane of the tubular cells. Both sugars compete for the same transport sites, indicating the sharing of a carrier (*Kleinzeller & McAvoy, J. Gen. Physiol. 62, 169, 1973*).

Using previously described experimental techniques, the following structural requirements for the interaction between these hexoses and the carrier were defined on the basis of an inhibition analysis employing structurally analogous sugars (5 mM) 1. A pyranose ring structure is essential, for D-galactitol did not inhibit the cellular uptake of both sugars. 2. A free hydroxl on C_1 is required since both - and -methyl-D-galactosides (-me-Gal), and -methyl-2-deoxy-D-galactoside was not transported into the cells. 3. While 2-deoxy-D-galactose (2-dGal) strongly inhibited the transport of D-galactose (Gal), 2-0-methyl-D-galctose (2-0-me-Gal) was not inhibitory. These observations indicate that a free hydroxal on C_2 prevents the interaction of the sugar with the carrier, suggesting a rather close packing between both molecules in the vicinity of C_2. 4. 3-Deoxy-D-galactose (3-dGal) was not inhibitory, thus demonstrating a requirement for a C_3-OH (in the axial configuration) 5. D-Glucose (Glc) did not inhibit the transport of both sugars. Thus, a hydroxl on C_4 in the axial configuration is mandatory. 6. L-Arabinose (L-Ara) and 6-deoxy-D-galactose (6dGal) did not inhibit the transport of either hexose. On the other hand, 6-0-methyl-D-galactose (6-0-me-Gal) had a marked inhibitory effect. These observations show that an oxygen on C_6 is required for an interaction between galactose or 2-deoxy-galactose and the carrier.

Figure 1 shows the results of the inhibition analysis of galactose transport at the antiluminal face of the flounder renal cells. Qualitatively identical results have been obtained using 2-dGal as model sugar. The model given in Figure 1 expresses the possible interaction between the sugar molecule and the carrier. In analogy to observations made in rabbit (*Kleinzeller, Proc. VIth Internat. Congr. Nephrology, Florence, 1975*) and flounder renal tissue (*Kleinzeller, Dubyak and Mullin, Bull. MDIBL #29 this issue*), it is assumed here that hydrogen bonds between the oxygen atoms at C_3, C_4, C_6 and the ring oxygen are involved in such interaction. the possibility that at C_1-OH the sugar molecule interacts with a phosphoryl group at the carrier is discussed elsewhere (*Dubyak and Kleinzeller, Bull. MDIBL #11 this issue*). This investigation was supported in part by