2R-9. Omeprazole inhibits the gastric pump but not the Na/K pump. Ouabain is the opposite – it inhibits the Na/K pump but not the gastric pump. Both inhibitors combine with the **extracellular** domains of their respective target proteins.

A. Ouabain (Drug B) should bind to parietal cells on the side facing:
(the lumen of the stomach) (the interstitial fluid) (either one) (neither – it needs to enter cells to act).

B. Omeprazole (Drug A) should bind to the parietal cells on the side facing:
(the lumen of the stomach) (the interstitial fluid) (either one) (neither – it needs to enter cells to act).

For an explanation of A & B, add the Na/K pump to the picture you drew for the previous problem. (Just show where the pump is – don't draw the complete structure. Assume it is a rectangle and put it in the proper place.) Then indicate where drug A (omeprazole) and drug B(ouabain) bind. Be sure you have the proper side of the proper pump.

C. People with reflux disease or ulcers take drugs to reduce stomach acid. Which of the following should be the best treatment? (ouabain – drug B) (Omeprazole – drug A) (both) (neither — try something else). Explain briefly.

2R-10. For each pump (H/K or Na/K), the alpha subunit contains the catalytic part of the protein and the part that binds the drugs. The alpha subunit is a single polypeptide with 10 transmembrane domains. Remember that the two pumps are homologous, and that the drugs bind to the extracellular domains of their respective pumps. A series of chimeric (recombinant) alpha subunits have been made. The chimeric alpha subunits contain parts from each pump protein. Some chimeric alpha subunits are sensitive to ouabain (inhibited) and some are resistant (not inhibited). The results are shown below. Answer the questions based on the experimental results. TM = transmembrane segment.

A. Which of the <u>following</u> part(s) of the normal **H/K** alpha subunit binds to ouabain? The part(s) between (TM 3 & 4) (TM 2 & 3) (TM 1& 2) (TM 3 & 4 and TM 1 &2) (none of these)
(all of these) (can't tell from information given).

B. Which of the part(s) of the normal **Na/K** alpha subunit that are listed bind(s) to ouabain? The parts between _____. (Same choices as in A.)

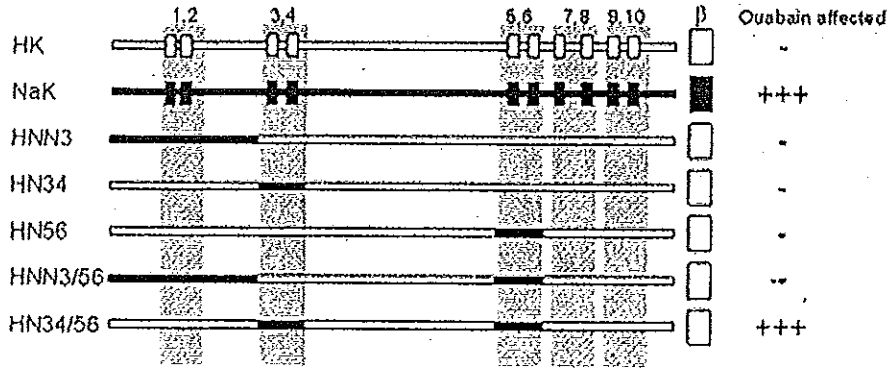
C. Where is the ***amino*** end of the catalytic subunit that makes up the Na/K pump?
(in the cytoplasm) (extracellular) (either way -- can't predict from information given).

D. The carboxyl end of the Na/K pump should be (on the same side as the amino end)
(on the opposite side) (either way -- can't predict from information given).

E. The carboxyl end of the Na/K pump and the carboxyl end of the H/K pump should be:
(on the same side) (on opposite sides) (either way -- can't predict from information given).

Show how the alpha subunit polypeptide of the ***Na/K*** pump is oriented in the membrane, and where ouabain (Drug B) binds in the normal protein. Indicate the ends of the protein, the TM's that are at or near the spot(s) where the drug binds, and both sides of the membrane.

F. If the alpha subunit of the Na/K pump is glycosylated, you are more likely to find sugars attached to (TM 3) (TM 2) (TM 1) (the region between TM 2 & TM 3) (the region between TM1 & TM 2)
(any of these TM regions) (the region between TM 2 & TM 3 and/or the region between TM 1 & TM 2) (any of these) (can't tell from info given).



Schematic representation of the chimeras and wild type alpha subunits. The top two bars represent the two normal alpha subunits. The remaining bars represent the chimeric proteins. The boxes represent the locations of the transmembrane sequences. The protein is written in the usual convention as to which end is the amino end. The *open regions of the bars* represent sequences from the H^+/K pump, and the *solid regions of the bars* represent sequences from the Na^+/K^+ pump. Inhibition by ouabain (+++), or lack of inhibition (-), is indicated at the *right.*
The figure is adapted from fig. 1 in Koenderink et al, PNAS *Oct. 10, 2000, vol. 97 no. 21, pp. 11209-11214.* The whole article, on which this problem is based, is at <u>http://www.pnas.org/content/97/21/11209.full</u>