Here's a different kind of question! Answering it requires you to read and understand two Abstracts of research presented at an annual meeting of the American Society for Cell Biology. By their very nature, such Abstracts are written in a terse, almost skeletal fashion and their comprehension requires the reader to make use of background information he or she has already mastered (and to read the Abstracts *carefully*)**.**

5. (30 pts) Much interest (and controversy) has developed in the past two decades concerning the "turnover" of the plasma membrane. How is this organelle synthesized, how is it degraded, and how are these two processes maintained in a steady-state balance? Our understanding of this phenomenon has been complicated by the observations that turnover may itself be part of such other important cellular processes as secretion (exocytosis) and both "feeding" and signalling (endocytosis). Consider this complex phenomenon in the light of what you have learned so far this term and the 2 sets of results abstracted below of work done **in the same laboratory**, and answer the following questions.

Binding and Internalization of GnRH by Rat Pituitary Gonadotrophs. **T.M. Duello, T.M. Nett*** and **M.G. Farquahar**, Section of Cell Biology, Yale University School of Medicine, New Haven, CT and Department of Physiology and Biophysics, Colorado State University, Fort Collins, CO.

Gonadotropin-releasing hormone (GnRH), like other peptide hormones, exerts its initial action in regulating secretion of gonadotropins (LH and FSH) by binding to specific plasma membrane receptors on the surface of the pituitary cells that secrete the LH and FSH. These cells are called gonadotrophs. Studies using fluorescent derivatives and ferritin conjugates of GnRH analogues have demonstrated their initial, diffuse binding to the cell surface followed by formation of aggregates which are gradually internalized by endocytosis. In this **in vivo** study we followed the fate of bound hormone by EM autoradiography using an ^{125}I-labeled analogue of GnRH. This analog was previously shown (Mol. Cell. Endocrinol. **19**: 601, 1980) to be taken up to a greater extent (~ 3-fold) and retained longer (~ 4-fold) by gonadotrophs than the native hormone. Ovariectomized rats were given intracarotid injections of 20 ng of the labeled analogue, and the pituitaries were fixed at 5, 15, 30, and 60 min. thereafter. A 1 min. pretreatment with a 200-fold excess of unlabeled analogue blocked binding at the 15, 30, and 60 min. intervals as demonstrated by very low cpm/pituitary gland and the absence of autoradiographic grains over gonadotrophs. The major findings were as follows: 1) At early time points ~50% of the grains were found within two half distances of the plasma membrane, the grain density decreasing over time; 2) the labeling of lysosomes was initially low and increased dramatically by 60 min.; 3) the grain density over the nucleus (<0.6), Golgi elements (<1.2), and rough ER (<1.2) remained relatively low at all time points; and 4) grain density of secretory vesicles was high (2.2-4.8) at all time points.

And -

Iodinated Cell Membrane Components Are Transported to the GolgiComplex. **P. Wilson*, D. Sharkey*, N. Haynes*, P. Courtoy* and M.G. Farquhar**. Section of Cell Biology, Yale University School of Medicine, New Haven, CT.

Recently it was shown that ferritin bound electrostatically to the plasma membrane of IgG-secreting murine myeloma cells is internalized by endocytosis and appears in the stacked Golgi cisternae and secretory vesicles after 60 min. (Ottosen et al, J. Exp. Med. **152**:1, 1980). In this study we covalently labeled plasma membrane components of cultured myeloma cells using the lactoperoxidase iodination (^{125}I) procedure of Hubbard and Cohn and followed the subsequent fate of labeled components by EM autoradiography. Characterization of the iodinated species revealed that ~15% of the label was incorporated into lipids and ~85% into proteins. Cellular incorporation of ^{125}I was a linear function of [^{125}I] in the medium. In a separate experiment it was shown that iodination did not affect significantly the rate of release of ^{14}C-labeled IgG (the normal secretory product of the myeloma). In 3 experiments EM autoradiographic analysis of cells fixed immediately after iodination at 4^{o}C showed up to ~80% of the total grains to be within two half distances (HD) of the plasma membrane and only 2% over the Golgi region. After 1 hour at 37^{o}C, 50-60% of the label remained associated with the plasma membrane and up to 23% was found in the Golgi region. Thus, 25-50% of the total internalized label was found in the Golgi region. Of the latter, the majority of the grains were found either directly over or within 2HD of the Golgi cisternae and secretory vesicles. At no time were > 1% of the total grains found over lysosomes. (Supported by USPHS Grant AM 17780.)

A. (8 pts) How are the fates of the plasma membrane in A. and B. being studied? Briefly describe each method and the component(s) of the plasma membrane that is (are) affected. Would you expect the methods would measure **identical** aspects of plasma membrane turnover? Why or why not?

B. (8 pts) Describe briefly the **results and the significance** of each experiment **taken individually.**

C. (8 pts) Discuss critically how both sets of results contribute to our understanding of plasma membrane turnover and of possible functions of the Golgi complex.

D. (6 pts) Describe one additional experiment that would provide useful information and describe clearly what the results would show.