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CHOLESTEROL SYNTHESIS: DETERMINATION OF SYNTHESIS RATE UTILIZING D_2O AND ISOTOPE RATIO MASS SPECTROMETRY. Abbie L. Esterman and Norman B. Javitt* Divsion of Hepatic Diseases, New York University School of Medicine, New York, N. Y. 10016.

Methods utilizing either ^3H_2O and/or HMG Co A reductase activity as an index of the cholesterol synthesis rate have limitations that led us to evaluate the use of D_2O. In preliminary studies it was found that Chinese hamster ovary (CHO) cells grown in media enriched with D_2O ranging from 10 to 40% yielded a distribution of deuterated cholesterol molecules corresponding to random incorporation of deuterium or hydrogen. Using 25% D_2O the preponderant species is M_O+6 (m/z=374), a peak easily distinguished from M_O and naturally abundant isotopes. Isolation of deuterated cholesterol from CHO cells grown in delipidated media permitted construction of a calibration curve of M_O/M_O+6 by addition of known amounts of cholesterol (M_O). Using an isotope ratio program the calculated and observed ratios were found to be linear from 1 to 100. Total cholesterol harvested from CHO cells grown in media containing fetal calf serum ranging from 0.1 to 4 % (cholesterol = 0.36 to 14.4 μg/ml) was found to have a progressive increase in the M_O/M_O+6 ratio indicating a decrease in newly synthesized cholesterol from 49 to 0.9%. Addition of 26-hydroxycholesterol (0.25μM) caused a reduction in both HMG Co A reductase activity and cholesterol synthesis rate. The method provides investigators with an easily applicable technique for the direct determination of absolute rates of cholesterol synthesis.

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Apolipoprotein Specificity of High Density Lipoprotein Receptors in Luteinized Rat Ovary. Jaulang Hwang and K.M.J. Menon. The University of Michigan, Ann Arbor, MI 48104. (Supported by NIH-HD 06556)

To determine which apolipoprotein is recognized by HDL receptor, Apo AI and Apo AII, the two major apolipoproteins in HDL, were purified from HDL by delipidation followed by successive chromatography until homogenity was established. These purfied apolipoproteiins were reconsitituted into dimyristoyl phosphatidyl choline vesicles (DMPC) and their ability to bind to rat luteal cell membranes was examined. Both ^{125}I-Apo AI•DMPC and ^{125}I-Apo AII•DMPC were shown to bind to ovarian membranes with Kd=2.87 and 5.70 ug protein/ml, respectively, and the binding of both ligands showed linear increase with inreasing membrane protein concentrations. The study of binding specificity showed that the binding of both ^{125}I-Apo AI•DMPC and ^{125}I-Apo AII•DMPC was inhibited by unlabeled Apo AI, Apo AI•DMPC, and HDL as well as by unlabeled Apo AII and Apo AII•DMPC, but not by DMPC vesicles alone, BSA or LDL. These results suggest that ^{125}I-Apo AI•DMPC and ^{125}I-Apo AII•DMPC bind to the same receptor and that their binding ability is conferred by their apolipoprotein moiety rather than the lipid environment. We have previously shown that the HDL receptor concentration in rat ovary was induced by in vivo hCG treatment. To test the inducibility of Apo AI and Apo AII binding to HDL receptor by hCG, rats were treated with 25 IU hCG and the isolated plasma membranes were examined to determine the binding affinity and capacity for labeled apolipoproteins. As expected, the binding sites for both ligands were induced by hCG treatment. These results further support our finding that the HDL receptor of luteinized rat ovary specifically recognizes both Apo AI and Apo AII and the binding is induced by hCG treatment.