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**20019** Incorporation of DOPA by Amelanotic Melanoma Cells: A Comparison of Data from Patient Biopsy, Soft Bilayer Agar Assay, and Xenograft. B. Persky*, F.L. Meyskens, Jr., and M.J.C. Hendrix, Dept. of Anatomy and Internal Medicine, Univ. of Arizona, Tucson, AZ. (Intr. by W.R. Ferris.)

The distinction between metastatic amelanotic melanoma and a poorly differentiated carcinoma is often difficult. With the use of the ultrastructural L-DOPA reaction as a tool for measuring tyrosinase activity within vesicular and cisternal organelles, cells containing melanin can be identified. Our study identified a tumor which was classified by light microscopy as an undifferentiated carcinoma and by electron microscopy as an undifferentiated neoplasm. Nine parameters were evaluated: a nude mouse xenograft, the patient's tumor cells in agar with and without L-DOPA treatment, and single cell suspensions of the patient's tumor cells with and without L-DOPA treatment. As controls, cells from Cloudman S91 (CCL) 53.1 melanoma, grown into colonies in agar with and without L-DOPA treatment and in single cell suspensions with and without L-DOPA treatment, were evaluated. The xenograft demonstrates no melanosomes. Cells in single cell suspensions with L-DOPA treatment and patient cells grown into colonies in agar with and without L-DOPA treatment contain granular dense bodies within the cytoplasm, suggestive of melanosomes. Premelanosomes (Stage II), Golgi, and Golgi-associated smooth endoplasmic reticulum contain DOPA-positive reaction product when cultured in the agar assay. A DOPA-positive reaction is also noted in the Golgi-associated smooth endoplasmic reticulum of the single cell suspensions. The CCL line demonstrates premelanosomes in all cases. These findings unequivocally identify the tumor as amelanotic melanoma and indicate that identification of DOPA-positive cells grown into colonies in agar are essential for diagnosis. These parameters may be applied to ambiguous cases of malignancy for which amelanotic melanoma is suspected. (Supported in part by grants from NIH (T32 CA09213 to B.P.) and ACS (PDT-184 to F.L.M. and PDT-205 to M.J.C.H.)

**20020** Coupling of Very Low Density Lipoprotein (VLDL) Apopeptide and Lipid Assembly and Secretion in the Intact Liver Cell. D.R. Janero, P. Siuta-Mangano*, and M.D. Lane*, Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21218 (Intr. by J.S. Addis).

Primary cultures of estrogen-induced chick liver cells have been used to investigate the relationships among VLDL apoprotein synthesis, the association and assembly of lipid with the VLDL apopeptides, and the secretion of the VLDL lipids. Hepatocyte monolayers in lipid-free medium were pulsed for 2.5 min with either [$^3$H]leucine or [$^3$H]palmitate and were then chased for 8.5 h with fresh lipid-free medium either containing 10 µM cycloheximide or not. At this concentration, cycloheximide inhibits cellular protein (and VLDL apoprotein) synthesis by >98% within 30 sec without impairing the secretion of VLDL apoprotein chains completed prior to the cycloheximide treatment. During the chase, parallel aliquots of media from both the [$^3$H]leucine- and [$^3$H]palmitate- labeled cultures were taken, and the VLDL was immunoprecipitated therefrom to assess the appearance of VLDL [$^3$H]apoproteins and [$^3$H]lipids in the medium. In both the absence and presence of cycloheximide, 3 h are required for ~95% of the pulse-labeled VLDL [$^3$H]apoprotein B to clear the cells and be secreted. Without cycloheximide, [$^3$H]triglyceride and [$^3$H]phospholipid specifically associated with VLDL apoproteins continue to be secreted over the course of the 8.5-h chase. The cycloheximide-treated cells do not secrete VLDL [$^3$H]lipids after 3 h into the chase-- i.e., after the cells have secreted their content of VLDL apoprotein synthesized prior to the cycloheximide block. The lipid secreted up to 3 h is associated with apopeptide chains completed prior to cycloheximide treatment but not yet secreted. We conclude that hepatic apoprotein synthesis is essential for the assembly and secretion of the VLDL acylglycerols and plays a decisive regulatory role in the control of VLDL biogenesis. Supported by NIH AM-06706 (DRJ), GM-00184 (PS), and AM-14574/5 (MDL).

**20021** Assembly and Secretion of Very Low Density Lipoprotein (VLDL) Apoproteins and Acylglycerols by the Intact Liver Cell in the Absence of Apopeptide Glycosylation. D.R. Janero and M.D. Lane*, Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21218.

The major and only glycosylated apoprotein of chick VLDL is apoprotein B; the "C" apopeptides are not glycosylated (*JCB, 73*: 332 [1977]). We have employed chick hepatocytes in primary culture to investigate the role of VLDL apopeptide glycosylation in the regulation of VLDL biogenesis. To this intent, hepatocyte monolayers were incubated for 4.5 hours in lipid-free medium either with or without 0.1 µg/ml tunicamycin, thereby, in the latter case, blocking further cellular protein (and apoprotein B) glycosylation and allowing secretion of all intracellular synthesized apoprotein B already glycosylated. The monolayers were then labeled for 2.5 h with [$^3$H]palmitate in fresh, lipid-free medium with or without tunicamycin, in accord with prior treatment. After labeling, the lipids specifically associated with secreted VLDL were isolated by immunoprecipitation and were analyzed chromatographically and radiochemically to quantify the [$^3$H]palmitate incorporated into the VLDL acylglycerols. The total amount of [$^3$H]palmitate, as well as its distribution among the various glycerolipid types, was identical in secreted VLDL containing either glycosylated or unglycosylated apoprotein B. In both cases, the "C" apopeptides were assembled into VLDL and were secreted normally. Fine-structural examination of the two types of VLDL particles revealed no morphological difference between them. These studies demonstrate that the state of apoprotein B glycosylation does not affect the assembly of VLDL apopeptides B and "C" with the major VLDL acylglycerols, the secretion of the lipids in specific association with the VLDL apoproteins, or the fine-structure of the VLDL particle. Thus, aglyco-apoprotein B can serve as a structural and functional component of hepatic VLDL. Supported by NIH AM-06706 (DRJ) and AM-14574/5 (MDL).

**20022** The Intracellular Distribution and Degradation of Immunoglobin G and Immuloglobin G Fragments Injected into HeLa Cells. S.W. Rogers, T. McGarry, R. Hough and M. Rechsteiner, Department of Biology, University of Utah, Salt Lake City, UT. (Intro. by T. Gurney).

Intact rabbit immunoglobulin G molecules (IgGs) and their papain or pepsin fragments were radioiodinated and injected into HeLa cells. Whole IgGs, Fab$_2$ and Fc fragments were degraded with half lives of 60-90 hours, whereas half lives of Fab fragments were 110 hours. These results indicate that proteolytic cleavage in the hinge region of the IgG molecule is not the rate limiting step in its intracellular degradation. The hingeless human myeloma protein, Mcg, was degraded at the same rate as bulk human IgG providing further evidence that the proteolytically susceptible hinge region is not important for intracellular degradation of IgG molecules. SDS acrylamide gel analysis of injected rabbit IgG molecules revealed that heavy and light chains were degraded at the same rate. Injected rabbit IgGs and rabbit IgG fragments were also examined on isoelectric focusing gels. Fab, Fab$_2$ and Fc fragments were degraded without any correlation to isoelectric point. Positively charged rabbit IgGs disappeared more rapidly than their negative counterparts contrary to the trend reported for normal intracellular proteins. The isoelectric points of two mouse monoclonal antibodies were essentially unchanged after injection into HeLa cells suggesting that the altered isoelectric profile observed for intact rabbit IgG resulted from degradation and not protein modification. The intracellular distributions of IgG fragments and intact rabbit IgG molecules were determined by autoradiography of thin sections through injected cells. Intact IgG molecules were excluded from HeLa nuclei whereas both Fab and Fc fragments readily entered them. Thus, for some proteins, entry into the nuclear compartment is determined primarily by size.



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