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OCULAR LENS GANGLIOSIDES IN SEVERAL SPECIES. C.P. Sarkar* and R.J. Cenedella. Kirksville Col. Osteo. Med., Kirksville, MO 63501

The present study was undertaken to clarify some confusion regarding the composition of gangliosides of the bovine ocular lens and also to compare the content and composition of lens gangliosides from different species. Windeler and Feldman (BBA, 202, 361, 1970), in contrast to Garg et al (Fed. Proc. 39, 2185, 1980), reported little ganglioside in bovine lens and observed the presence of only one ganglioside (GM_3). In the present study gangliosides from normal beef, pig and rat lens plus human cataractous lens were extracted by either the Folch-Suzuki Partition (FSP) or the modified tetrahydrofuran method (THF). The sialic acid content of ganglioside and protein fractions was separately measured by colorimetric assay. Bovine lens was found to contain 17.4 µg of ganglioside sialic acid/g tissue (wet wt) by the FSP method and 24.8 µg/g by the THF method. These values are similar to those reported by Garg et al. Pig, rat and human lens, extracted by the FSP procedure, respectively contained 14.4, 60.2 and 260.4 µg of ganglioside sialic acid/g lens (wet wt). No protein-bound sialic acid was measured in any lenses. Bovine and pig lens gangliosides were fractionated by thin-layer chromatography and the percent distribution of sialic acid determined.

	% Total Ganglioside-Sialic Acid			
	GM_3	GM_1	GD_{1a}	GT_1
Beef lens	73	6	17	4
Pig lens	68	10	15	7

Studies are continuing to more fully characterize these potentially important membrane constitutents of the ocular lens. (Supported by NIH Grant EY02568).

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COMPARISON AND GANGLIOSIDE COMPOSITION OF METASTATIC AND NON-METASTATIC TRANSPLANTABLE TUMORS FROM THE JAW REGION OF THE RAT. Gary R. Matyas* and Dorothy A. Werderitsh* (SPON: T. W. Keenan). Purdue Univ., W. Lafayette, IN. 47907.

Two rat tumors, a spontaneous jaw tumor (WJT) which has 100% metastatic incidence and a jaw tumor (JT_2TS_P) which has not metastasized in 24 generations were compared. Both tumors were white in color, ovoid in shape and well circumscribed. The non-metastatic JT_2TS_P tumor line has a growth rate of 0.17 cm/day, while the metastatic WJT tumor line has a growth rate of 0.017 cm/day. Upon histological examination, both tumors show morphological features characteristic of squamous cell carcinomas. Ultrastructurally both tumors contained poorly developed Golgi apparatus, sparse rough endoplasmic reticulum, bizarre mitochondrial forms, irregularly shaped nuclei and abundant lysosomes. In general, the lysosomes appeared to be more abundant in the metastatic line. Total ganglioside sialic acid was approximately 3 times greater in the metastatic than the nonmetastatic line. Thin layer chromatography indicated an absence of the gangliosides which co-migrated with G_{D1b} and G_{T1b} in the metastatic tumor line. Recently the gangliosides G_{D1a}, G_{D1b} and G_{T1} have been shown to bind fibronectin in vitro. The loss of the ability of cells to bind fibronectin is strongly correlated with metastasis. This suggests that the loss of the complex fibronectin-receptor gangliosides may contribute to the metastatic potential of the WJT tumor line. (Supported in part by grants from ACS CD-52 and NIH CA 18801).

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AN ACUTE HUMAN LEUKEMIA CONTAINING BOTH GLOBO- AND NEOLACTO-TYPE NEUTRAL GLYCOSPHINGOLIPIDS. W.M.F. Lee,* B.A. Macher* and J.C. Klock* (SPON: D. Papahadjopoulos). Cancer Research Institute, Univ. of California, San Francisco, CA 94143

We analyzed neutral glycosphingolipids from the cells of a patient with acute myelomonocytic leukemia. The leukemic cells had morphologic and cytochemical characteristics of both myeloid and monocytic cells. The glycosphingolipids were analyzed by thin layer, gas-liquid and high pressure liquid chromatography, and endo-ß-galactosidase treatment. These cells contained two tetrahexosylceramides, globotetraosylceramide and lactoneotetraosylceramide. Our earlier analyses of human leukocyte neutral glycosphingolipids revealed that neutrophils and chronic myelogenous leukemia cells contain certain compounds of the neolacto series with some gala-type compounds. In contrast, peripheral blood lymphocytes and chronic lymphocytic leukemia cells contain compounds of the globo series. No leukocytes previously studied have been found to contain both globo and neolacto compounds; thus, our patient's leukemia cells represent the first leukocyte population found to have both these classes of glycosphingolipids. As the structures of the neutral glycosphingolipids of monocytic cells are unknown, the presence of globotetraosylceramide may represent monocytic differentiation of the myelomonocytic leukemia cells. On the other hand, its presence may reflect leukemic transformation of myeloid and/or monocytic cells with subsequent alteration in glycosphingolipid metabolism.

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A NOVEL PHENOLIC GLYCOLIPID FROM *MYCOBACTERIUM LEPRAE*. Shirley W. Hunter* and Patrick J. Brennan. CSU, Fort Collins CO 80523 and Natl. Jewish Hosp., Denver, CO 80206

A phenolic glycolipid (mycoside) was obtained in high amounts (2% of dry wt.) from *M. leprae* isolated from infected armadillo liver. IR and NMR spectroscopy showed that the *M. leprae* glycolipid is closely related to mycoside A from *M. kansasii* and is therefore a glycosylphenolic phthiocerol diester. The crucial difference between the two products is in the composition of the attached trisaccharide. GLC-MS showed that the product from *M. kansasii* is composed of 2,4-di-O-Me-Rha, 2-O-Me-Rha and 2-O-Me-Fuc, whereas that from *M. leprae* contains a 2,3-di-O-Me-6-deoxyhexose, 3-O-Me-Rha and a 3,6-di-O-Me-hexose. The distinct composition of the oligosaccharide segment of the glycolipid from *M. leprae* may be useful for chemical and serological differentiation of this organism from other mycobacteria. High amounts of the glycolipid were also found in infected liver residue freed of *M. leprae*, suggesting that it may be responsible for the electron-transparent "foam" surrounding the organism in infected tissue. (Supported by NIH Contract AI-92625)

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URINARY GLYCOSPHINGOLIPIDS IN FAMILIAL HYPERCHOLESTEROLEMIA. S. Chatterjee, C. Sekerke*, and P.O. Kwiterovich, Jr.* Dept. Pediatrics, Johns Hopkins Univ., Baltimore, MD 21205.

Glycosphingolipids (GSL) were isolated from the urinary sediment (24 hr specimen) of 8 normal humans, a subject with Fabry's disease and 5 homozygotes with familial hypercholesterolemia (FH) and the content of GSL determined by GLC. Trace amounts of GL_{1a}, GL_{1b}, GL_2, GL_3 and GL_4 were found in normal urine sediment. The levels of GL_2 and GL_3 in the sediment from Fabry's urine were 40-50 fold higher than normal. GL_{1a}, GL_{1b} and GL_2 were the predominant GSL in the urine sediments from FH homozygotes. In 4 of 5 FH homozygotes, the urine sediment contained an increase (nmole glucose/24 hr) in GL_{1a} (2-7 fold), GL_{1b} (1.2-3 fold) and GL_2 (4-27 fold). The other FH homozygote (GM 2000) who had undergone a portacaval shunt had GSL that were below normal. When the data were expressed as nmole glucose/mg protein/24 hr or nmole glucose/ug cholesterol/24 hr, the content of GL_2 was elevated 3-53 fold and 7-9 fold, respectively. One FH homozygote (T.B.) underwent plasma exchange therapy that produced lower plasma and urine levels of GL_2; however, 10 days after exchange, the urinary GSL levels approached pre-exchange levels. These data suggest that there is an abnormality of GSL metabolism associated in the FH and that the excretion of GSL in urinary sediment can be modified with treatment.

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CHARACTERIZATION OF LIPID-LINKED OCTA-, NONA- AND DECASACCHARIDES INVOLVED IN BIOSYNTHESIS OF MAMMARY GLYCOPROTEINS. Gary Perdew* (SPON: R. F. Davis) University of Maryland, College Park, Md. 20742.

Earlier we reported (Vijay and Perdew, J. Biol. Chem. 255, 11221-11226 (1980)) the presence of isomers in lipid-linked hexa- and heptasaccharides during the in vitro biosynthesis of asparagine-linked glycoproteins by the lactating bovine mammary tissue. Using GDP-[^{14}C]mannose as the precursor, the octa-, nona- and decasaccharide intermediates have been isolated. These have the general structure Man_n-GlcNAcβ1→4(3)GlcNAcβ1→4(3)GlcNAc. The acetolysis data indicate that two or more isomers are present in each of these saccharides. Besides Manα1→3Man, Manα1→2Manα1→3Man and Manα1→2Manα1→3(Manα1→6)Man linkedαl→6 to the β-mannosyl residue in Manα1→2Manα1→2Manα1→3Manβ1→4GlcNAcβ1→4GlcNAc in the octa-, nona- and decasaccharide, respectively, as in Chinese hamster ovary cells, there also appear to be saccharides of the structure: Manα1→2Manα1→3[$(Man_n$ α 1→) Manα1→6] Manβ1→4GlcNAcβ1→4GlcNAc, n = 2 to 4. Methylation analysis is currently being conducted to delineate the inter-residue linkages for the Man_nα1→ portion. Studies with only UDP-[^{3}H]GlcNAc as the precursor to confirm the data with [^{14}C] mannose labeling, will also be presented. (Supported by N.I.H. Grant AM19682 and R.C.D.A. AM452 to I.K.V.).

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NEW GANGLIOSIDES FROM HUMAN ERYTHROCYTES. S. K. Kundu*, D. M. Marcus, I. Pascher* and B. E. Samuelsson*. Baylor College of Medicine, Houston, Tx. 77030 and Univ. of Goteborg, Sweden.

We have purified nine gangliosides from human erythrocytes that have not been previously identified in these cells and three of the gangliosides appear to be new compounds. The structures of these compounds were tentatively established on the basis of sugar composition, neuraminidase treatment, methylation, partial acid hydrolysis and mass spectrometry. The tentative structures of two of these compounds are:

NeuAc(α2-3)Gal [(ß1-4)GlcNAc(ß1-3)Gal] $_3$(ß1-4)Glc-Cer

NeuAc(α2-3)Gal(ß1-4)GlcNAc(ß1→6 / →3)Gal(ß1-4)GlcNAc(ß1-3)Gal(ß1-4)Glc-Cer

The neutral portion of a disialo compound appears to have the following sequence:

Gal-GalNAc-Gal-Gal-Glc-Cer

Other gangliosides identified include G_{M2}, G_{M1}, G_{D3}, G_{D1a}, G_{D1b} and disialosyllactoneotetraosylceramide.

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VITAMIN B_6 UPTAKE, METABOLISM, AND BINDING TO PROTEINS IN HUMAN ERYTHROCYTES. Margaret L. Fonda and Candace W. Harker.* Univ. of Louisville Health Sci. Ctr., Louisville, KY 40292.

Vitamin B_6 is actively taken up by erythrocytes and metabolized to its physiological forms. The rate of uptake of pyridoxine and the distribution of the metabolites in erythrocytes were determined by incubating isolated human erythrocytes in isotonic phosphate with [^{3}H]pyridoxine. After 60 min at 37°, the erythrocytes had taken up 70-80% of the radioactivity, most of which (90%) was in the form of pyridoxal-P. At least 99% of the radioactivity taken up by the erythrocytes was in the soluble fraction of the cells. Approximately 60% of the radioactivity was protein bound. To identify the proteins to which the radioactive B_6 was bound, the hemolysate was fractionated by chromatography on DEAE Sephadex and CM cellulose. The protein fractions containing radioactivity were analyzed further by chromatography on Sephadex G-150. Most of the radioactive B_6 was bound to hemoglobin while only 5% of the radioactive B_6 was bound to aminotransferases in human erythrocytes. To determine the form of vitamin B_6 bound to hemoglobin, the radioactive hemoglobin fraction was treated with trichloroacetic acid and heat. The extract was analyzed by chromatography on Amberlite CG-120 and DE-32 cellulose. Most of the radioactive B_6 was pyridoxal-P. In conclusion, erythrocytes take up and concentrate pyridoxine. The pyridoxine is rapidly metabolized, primarily to pyridoxal-P. Much of the newly synthesized pyridoxal-P is bound to hemoglobin. (Supported in part by NIH Grant AM 195707).

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STRUCTURE OF THE ß SUBFORM OF CYTOSOLIC ASPARTATE AMINOTRANSFERASE. Carol M. Metzler*, Hiroshi Ueno*, Jennifer Morris*, Kurt Timmerman*, Robert D. Scott*, and David E. Metzler, Iowa State Univ., Ames, IA 50011 and Paul H. Rogers*, Patrick D. Briley*, and Arthur Arnone, Univ. of Iowa, Iowa City, IA 52242.

One subunit of the ß form of the dimeric (MW 92,700) cytosolic aspartate aminotransferase of pig hearts is inactive and has an absorption band at 340 nm rather than at the 362 nm of the active subunit. Although pyridoxal phosphate is released at pH 12 it cannot be removed reversibly at lower pH as it can be from the active subunit. Crystals of the ß subform are isomorphous with those of the fully active α subform whose structure is being determined (Arnone et al.). A difference electron density map shows that the inactive subunit contains something resembling a normal substrate in the active site. Denaturation by heating at pH 7 releases a low molecular weight compound resembling pyridoxamine phosphate in spectral properties but more negatively charged and unreactive with ninhydrin. The electrophoretic mobility at pH 6 is similar to that of pyridoxal phosphate. Treatment with alkaline phosphatase causes a decrease in mobility as expected for dephosphorylation. The compound has been purified by ion exchange chromatography and the structure is being determined. The compound may represent the product of a natural mechanism-based inhibitor or a true substrate which has reacted in an abnormal way. (Supported in part by USPHS NIH Grants AM01549 and AM17563.)

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OPTIMIZED EXTRACTION AND DIFFERENTIAL DETERMINATION OF PYRIDOXAL AND PYRIDOXAMINE PHOSPHATES IN RAT TISSUES. Ashok K. Sawhney* and Bob In-yu Yang* (SPON: Paul E. Blatz). Department of Chemistry, College of Arts and Sciences and School of Medicine, University of Missouri, Kansas City, MO 64110.

Rat blood plasma, liver and brain were treated with either sodium hyroxide or trichloroacetic acid. Optimal conditions for liberating pyridoxal-P and pyridoxamine-P were as follows. For liver and brain, tissue homogenate was made 1N in NaOH and heated in boiling water for 30 min. Following neutralization and addition of EDTA, the vitamers were determined employing the apoenzyme of pig heart aspartate aminotransferase. Blood plasma was similarly processed, but without heat treatment which reduced the yield considerably. Addition of EDTA increased assyable vitamers in tissue extracts. Recovery of authetic vitamers added to tissue extracts was greater than 90%. Extraction with 0.65 M trichloroacetic acid liberated lower quantities of the vitamers from all tissues. Femtomole sensitivity of the assay permitted 240, 20,000, and 6,000-fold dilution of blood plasma, liver and brain, respectively. Differential determination of pyridoxal-P was performed by first measuring the sum of the two vitamers, both of which activate the apoenzyme. In situ reduction of pyridoxal-P was obtained by addition of sodium borohydride at 2.5 mM in 20 mM sodium hydroxide. Excess reagent was destroyed with acetone. Pyridoxamine-P which remained undiminished in the reaction mixture was assayed. The difference yielded pyridoxal-P. (Supported by USDA SEA 59-2291-0-1474-0)

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VITAMIN B-6 METABOLISM IN REGENERATING RAT LIVER AND FAST-GROWING MORRIS HEPATOMAS. John W. Thanassi, Louise M. Nutter* and Natalie T. Meisler* Univ. of Vermont, Burlington, VT 05405

The activities of vitamin B-6 metabolizing enzymes were measured in two fast-growing Morris hepatomas and compared with the activities found in host livers, control livers and regenerating livers. In addition, ornithine decarboxylase (ODC) and pyridoxal phosphate (PLP) levels were determined. Rats employed in this study were maintained on both pyridoxine (PN)-sufficient and deficient diets. ODC and pyridoxine phosphate (PNP) phosphatase activities in hepatomas and regenerating liver were elevated when compared to control and host livers. Liver PN kinase activity was higher in rats maintained on the PN-sufficient diet. In contrast, PN kinase activities in hepatomas were unaffected by PN intake and were significantly lower than in host or control livers. Partial hepatectomy had no effect on liver PN kinase activity. PNP oxidase activities in the livers of control and tumor-bearing animals were not significantly different from each other nor were they affected by dietary PN. Hepatomas, on the other hand, had little or no PNP or PN oxidase activity. Regardless of the nutritional status, hepatomas had lower PLP levels than host or control livers. These data indicate that fast-growing Morris hepatomas differ significantly from control and regenerating rat livers in that they appear incapble of the complete synthesis of PLP from precursor vitamer forms such as PN. (Supported by NIH Grant # AM-25490).