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Biochemistry and nutrition

INFLUENCE OF CHOLESTEROL ON THE ROTATION AND SELF-ASSOCIATION OF BAND 3 IN THE HUMAN ERYTHROCYTE MEMBRANE. T. Muhlebach and R. J. Cherry (Eidgenossische Technische Hochschule, Laboratorium fur Biochemie, ETH Zentrum, CH 8092 Zurich, Switzerland) *Biochemistry 21* (18): 4225-4228 (1982) The cholesterol/phospholipid mole ratio (C/P) in the human erythrocyte membrane was varied by incubating cells with liposomes. The rotational mobility of band 3 proteins was measured in these membranes by observing flash-induced transient dichroism of the triplet probe eosin maleimide. Measurements were performed with membranes in which associations of band 3 with cytoskeletal proteins were removed by mild proteolysis with trypsin. It was found that decreasing C/P resulted in a more rapid decay of the flash-induced anisotropy. The anisotropy decay curves were analyzed by curve-fitting procedures, which indicated the existence of different sized small aggregates of band 3. The changes in the decay curves with varying C/P can be explained by an effect of cholesterol on the size distribution of these aggregates. The experiments suggest a possible role of cholesterol in regulating associations between integral membrane proteins.

STIMULATION OF COLONIC SECRETION BY LIPOXYGENASE METABOLITES OF ARACHIDONIC ACID. M.W. Musch, R. J. Miller, M. Field, and M.I. Siegel (Department of Medicine University of Chicago, Chicago, Illinois 60637)*Science 217* (4566): 1255-1256 (1982). Both 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and 5-hydroxyeicosatetraenoic acid (5-HETE) increased the short-circuit current (I_{sc}) in rabbit colonic mucosa mounted in vitro in Ussing chambers. Measurements of chlorine-36 fluxes indicated that the I_{sc} response to 5-HPETE is due to stimulation of active chlorine secretion. 9-, 11-, and 12-HPETE's and leukotrienes C_4 and B_4 produced either very small increases in I_{sc} or no increase. In contrast to results in rabbit colon, no HETE or leukotriene was effective in rabbit ileal mucosa. The effects of 5-HPETE in the rabbit colon were unaffected by mepacrine, but could be partially blocked by indomethacin. These results suggest that drugs which block both cyclooxygenase and lipoxygenase may be effective antidiarrheals in patients with colitis.

EFFECTS OF DIETARY PROTEIN AND AMINO ACIDS ON THE METABOLISM OF CHOLESTEROL-CARRYING LIPOPROTEINS IN RATS. M.-S.C. Park and G. U. Liepa (Dept. of Nutrition and Food Sciences, Texas Woman's University, Denton, TX 76204) *J. Nutr. 112* (10): 1892-1898 (1982). The effect of various dietary proteins and amino acids on serum lipid metabolism was studied by using male Sprague-Dawley rats. A stock diet containing casein as a protein source was fed to control animals, whereas a vegetable protein diet (cottonseed based) was fed to one experimental group. Two other experimental diets were formulated to determine if the amino acid ratios in the proteins played a role in the alteration of serum cholesterol levels. One of these diets contained casein plus enough additional arginine to make its arginine-to-lysine ratio similar to that found in cottonseed protein. The other diet contained cottonseed protein plus enough lysine to duplicate the arginine-to-lysine ratio of casein. Rats fed a diet containing protein from animal sources had greater serum and high-density lipoprotein (HDL)-cholesterol concentrations as well as increased lecithin:cholesterol acyltransferase (LCAT,EC2.3.1.43) activities than those which had been fed a diet containing protein from plant sources. Animals fed arginine-supplemented casein diet showed a decrease in both serum and HOL: cholesterol when compared to the casein control group, whereas the addition of lysine to cottonseed protein diet caused an increase in the same two cholesterol fractions.

EFFECT OF SELENIUM AND VITAMIN E DEFICIENCY OF NITROFURANTION TOXICITY IN THE CHICK. F. J. Peterson, G.F. Combs, Jr.,J.L. Holtzman, and R.P. Mason (Department of Poultry and Avian Sciences, Division of Nutritional Sciences, Rice Hall, Cornell University, Ithaca, NY 14853) *J.Nutr. 112* (9): 1741-1746 (1982). The acute toxicity of nitrofurantion was studied in the young chick deficient in selenium (Se) and/or vitamin E (E). This new and potentially valuable animal model proved to be very sensitive to the toxicity of this nitro drug. The 48-hour LD_{50} for nitrofurantoin decreased from 148 mg/kg in the Se- and E-supplemented chicks to 53 mg/kg in the Se and E-deficient chicks. The addition of Se (0.10 ppm as Na_2SeO_3) alone, but not E (100 IU/kg diet as dl-α-tocopheryl acetate) reduced the toxicity of nitrofurantoin, so that the LD_{50} for the chicks given Se alone was the same as the LD_{50} for the E- and Se- fed chicks. Se and E deficiency significantly decreased the Se-dependent glutathione peroxidase and the plasma tocopherol levels. Hepatic glutathione content, hepatic catalase and superoxide dismutase were unchanged by the dietary treatments. However, a toxic dose of nitrofurantoin significantly decreased hepatic glutathione content over time. These data supported the concept that the toxicity of this drug may be mediated in part by an oxidative stress generated by the futile reductive metabolism of the parent compound.

INTERACTION BETWEEN 1,25-DIHYDROXYVITAMIN D_3 RECEPTORS AND INTESTINE NUCLEI. BINDING TO NUCLEAR CONSTITUENTS IN VITRO. J.W. Pike (Dept. of Biochem., Arizona Health Sci. Center, Univ. of Arizona, Tucson, AZ 85724) *J. Biol. Chem* 257 (12): 6766-6775 (1982). The molecular action of 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3O$ is thought to involve its localization within the nucleus of target cells, a process mediated by intracellular receptors. This report probes both the association between chick intestinal $1,25(OH)_2D_3$ receptors and purified homologous nuclei and the interaction between this receptor and nucleic acids. $1,25(OH)_2D_3$ receptors bound to purified nuclei in an apparently saturable manner (K_d=2.2-4.8×10^{-10}M) under conditions of intermediate ionic strength and constant protein concentration.

THE EFFECT OF BILE ACID ON LIPID AND NITROGEN RETENTION, CARCASS COMPOSITION, AND DIETARY METABOLIZABLE ENERGY IN VERY YOUNG CHICKS. D. Polin and T.H. Hussein (Dept. of Animal Sci., Michigan State Univ., East Lansing, MI (48824) *Poultry Sci. 61* (8): 1697-1707 (1982). Heavy breed chicks fed isonitrogenous and isocaloric diets containing 7.6% tallow or one of four different hydrogenated animal and vegetable fats (HAVFs) at 6.2% retained 25% less lipid when 1 week of age than when 2 or 3 weeks of age. The addition of .04% sodium taurocholate (NaT) improved lipid retention most at 1 week of age but had no effect on retention of nitrogen. The apparent metabolizable energy (ME_a) was 10% less for the chick 1 week of age, reflecting lower lipid and nitrogen retentions by the very young chicken. Percentages of carcass lipid and protein were lowest at 1 week of age (10.4%) and increased by the 3rd week (17%). The addition of NaT did not cause any significant changes in carcass composition. The data indicated that absorptive mechanisms for lipid and protein are not fully developed in the very young chick and that dietary bile salts tend to improve lipid but not nitrogen absorption.

PHOSPHATIDYLCHOLINE-PROMOTED INTERACTION OF THE CATALYTIC AND REGULATORY PROTEINS OF ADENYLATE CYCLASE. E.M. Ross (Dept. of Pharmacology, Univ. of Texas Health Science Center, Dallas, TX 75235) *J. Biol. Chem. 257* (18):10751-10758 (1982). The catalytic protein of rabbit hepatic adenylate cyclase, after chromatographic separation from the GTP-binding regulatory protein (G/F) is essentially free of endogenous phospholipids. This preparation is active in the presence of MN^{2+} and is markedly stimulated by forskolin, but it is stimulated only slightly by the addition of purified G/F plus an activator. The ability of activator-liganded G/F to stimulate the activity of the resolved catalyst is increased up to 8-fold by the addition of either dimyristoylphosphatidylcholine or several other phosphatidylcholines. Phosphatidylcholine stabilizes the catalyst to denaturation but has little effect on its basal activity or on its stimulation by Mn^{2+} or forskolin. It also had no stimulating effect on the activation of G/F by GTPγS. These data are interpreted as showing that phosphatidylcholine promotes or is required for the stimulatory interaction of activatorliganded G/F with the catalytic protein of adenylate cyclase. Lubrol 12A9, Triton X-100, cholate, lysophosphatidylcholine, digitonin, and phosphatidylserine could not substitute for phosphatidylcholine. The detergents inhibited

stimulation by liganded G/F even in the presence of phosphatidylcholine. Removal of cholate from a mixture of soluble catalytic protein and phosphatidylcholine by dialysis and surcrose density gradient centrifugation caused the binding of catalytic protein to large unilamellar vesicles. This preparation was further reconstituted with increasing amounts of G/F to yield vesicles with varied G/F: catalyst ratios and similarly varied responses to G/F mediated activating ligands.

ENERGY BALANCE AND MITOCHONDRIAL FUNCTION IN LIVER AND BROWN FAT OF RATS FED "CAFETERIA" DIETS OF VARYING PROTEIN CONTENT. N.J. Rothwell, M.J. Stock, and R.S. Tyzbir (Dept. of Phsiol., St. George's Hosp. Med. Schl. Tooting, London SW17 ORE, U.K.) *J. Nutr. 112* (9): 1663-1672 (1982). Rats fed 'cafeteria' diets with low (7%, LP) normal (23%, NP) or high (33%, HP) protein contents showed increases in metabolizable energy intake (kJ/kg$^{0.75}$, 23-41%) and in energy expenditure (36%) compared to controls fed stock diet (27% protein). The high metabolic rates were inhibited by β-adrenergic blockade with propranolol. All rats fed cafeteria diets deposited more fat than controls, but the LP diet depressed growth, and these animals also showed the lowest energetic efficiency. Brown adipose tissue (BAT) mass and protein content were increased in all groups fed cafeteria diets, but the largest changes occurred in LP-fed animals, and the smallest in the HP group. Hepatic mitochondrial α- glycerophosphate shuttle activity and plasma triiodothyronine levels were elevated two-fold in rats fed LP cafeteria diet compared to controls, but the other cafeteria diet groups showed little or no changes, and shuttle activity in BAT was not affected by any of the diets. Blood glucose and plasma insulin levels were similar for control, NP and HP animals, whereas glucose levels were slightly lower and insulin levels were very much lower in the rats fed LP cafeteria diet.

RECIPROCAL EFFECT OF APOLIPOPROTEIN C-II ON THE LIPOPROTEIN LIPASE-CATALYZED HYDROLYSIS OF *p*-NITROPHENYL BUTYRATE AND TRIOLEOYLGLYCEROL. K. Shirai, R.L. Jackson, and D.M. Quinn (Division of Lipoprotein Research, Departments of Pharmacology and Cell Biophysics and Biological Chemistry, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267) *J. Biol. Chem.* 257(17): 10200-10203 (1982). Interaction of purified bovine milk lipoprotein lipase (LpL) with sonicated vesicles of dipalmitoyl phosphatidylcholine in the gel phase is associated with an increase in the rate of the LpL catalyzed hydrolysis of *p*-nitrophenyl butyrate. There is a 6-fold increase in V_{max}. Apolipoprotein C-II, the activator protein for LpL, inhibits the LpL-catalyzed hydrolysis of *p*-nitrophenyl butyrate. With 0.5 mol % tri[^{14}C] oleoylglycerol present in the dipalmitoyl phosphatidylcholine vesicles and in the presence of 20 mM Ca^{2+}, the rate of *p*-nitrophenyl butyrate hydrolysis is decreased reciprocally compared to trioleoylglycerol hydrolysis and is dependent on apolipoprotein C-II. These results suggest that apolipoprotein C-II enhances the activity of LpL by increasing the affinity of the active site of LpL for triacylglycerol.

LATERAL DIFFUSION OF UBIQUINONE DURING ELECTRON TRANSFER IN PHOSPHOLIPID-AND UBIQUINONE-ENRICHED MITOCHONDRIAL MEMBRANES. H. Schneider, J.J. Lemasters, and C.R. Hackenbrock (Labs. for Cell Biol., Dept. of Anatomy, Schol. of Med., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27514) *J. Biol. Chem. 257* (18): 10789-10793 (1982). After fusion of small unilamellar phospholipid liposomes with mitochondrial inner membranes, the rate of electron transfer between membrane dehydrogenases and cytochrome c decreases as the average distance between integral membrane proteins increases, suggesting that electron transfer is mediated through a diffusional process in the membrane plane (Schneider, H., Lemasters, J.J., Hochli, M., and Hackenbrock, C.R. (1980)., *J. Biol. Chem. 255*, 3748-3756). The role of ubiquinone in this process was evaluated by fusing liposomes containing ubiquinone-10 or ubiquinone-6, with inner membranes. In control membranes enriched with phospholipid only, ubiquinol-cytochrome *c* reductase and NADH- and succinate-cytochrome *c* reductase activities decreased proportionally to the increase in bilayer lipid. These decreases were restored substantially in phospholipid plus ubiquinone-supplemented membranes. The degree to which restoration occurred was dependent upon the length of the isoprenoid side chain of the ubiquinone with the shorter chain length ubiquione-6, always giving greater restoration that ubiquinone-10. It is concluded that electron transfer between flavin-linked dehydrogenases (Complexes I and II) and cytochrome *bci* (Complex III) occurs by independent, lateral diffusion of ubiquinone as well as the protein complexes within the plane of the membrane.

AMP DEAMINASE AS A CONTROL SYSTEM OF GLYCOLYSIS IN YEAST. MECHANISM OF THE INHIBITION OF GLYCOLYSIS BY FATTY ACID AND CITRATE. M. Yoshino and K. Murakami (Dept. of Biochem., Yokohama City Univ. Schl. of Med., Yokohama 232 and the Dept. of Lab. Med., St. Marianna Univ. Schl. of Med., Kawasaki 213, Japan) *J. Biol. Chem. 257* (18): 10644-10649 (1982). The role of fatty acid and citrate on the interaction of the AMP deaminase (EC 3.5.4.6) reaction with glycolysis was investigated using permeabilized yeast cells. (a) Linolenate and citrate inhibited glycolytic flux and the recovery of the adenylate energy charge; however, linolenate remarkably retarded the depletion of the total adenylate pool, which was not at all affected by the addition of citrate. (b) Linolenate inhibited AMP deaminase activity in situ, resulting in the subsequent decrease in ammonium production, which reduced the activity of 6-phosphofructokinase (EC 2.7.1.11), whereas linolenate itself had no ability to inhibit the phosphofructokinase activity in the presence of excess ammonium concentration. (c) Citrate inhibited the activity of phosphofructokinase in situ in the presence and absence of ammonium ion, followed by an inhibition of glycolysis; however. AMP deaminase activity was not inhibited by citrate. The inhibition of glycolysis by fatty acids can be accounted for by the lowered activity of phosphofructokinase as a result of the decreased level of ammonium ion through the inhibition of the AMP deaminase reaction by these ligands, whereas the effect of citrate on glycolysis is a direct inhibition of phosphofructokinase without affecting the activity of AMP deaminase. Fatty acid and citrate, a principal metabolic product of fatty acid oxidation, can be responsible for the control of glycolysis in two different manners.

EFFECT OF DIETARY POLYUNSATURATED FATTY ACIDS ON THE ACTIVITY AND CONTENT OF FATTY ACID SYNTHETASE IN MOUSE LIVER. R.S. Schwartz and S. Abraham (Bruce Lyon Memorial Res. Lab., Children's Hospital Medical Center, 51st and Grove Streets, Oakland, CA 94609) *Biochim. Biophys. Acta 711* (2): 316-326 (1982). When mice, previously fed a standard laboratory mouse chow diet, were fed a high carbohydrate (50% glucose) diet, the activity of hepatic fatty acid synthetase per mg cytosolic protein increased approximately 3-fold over an 11-day period. However, when mice were placed on an isocaloric diet the specific activity of the enzyme did not increase above the chow-fed levels. Using antibody prepared against pure mouse liver fatty acid synthetase, we showed that the increase in the specific activity of fatty acid synthetase in the high carbohydrate-fed animals resulted from an elevation in the hepatic content of the enzyme. This increase was a result of (a) an increase in the rate of synthesis of the enzyme relative to that of total protein and (b) a decrease in the enzyme's degradative rate, when compared to these parameters measured in the livers of the isocaloric-fed animals. Futhermore, these dietary-induced changes in enzyme specific activity were not accompanied by changes in the catalytic efficiency of fatty acid synthetase, since both diet-fed animals showed identical immunoequivalences and contained similar amounts of immunoprecipitable ^{3}H-labeled enzyme protein per unit enzyme activity. The results of experiments in which we administered pure fatty acids to mice maintained on a 50% glucose diet suggested that the ability of a fatty acid to inhibit hepatic fatty acid synthetase activity and to prevent an increase in hepatic fatty acid synthetase protein was related to the degree and position of unsaturation of the fatty acid administered and not to the ability of the fatty acid to act as prostaglandin precursor. Those 18-carbon fatty acids which possessed a double bond at postitions Δ9,12 were the most effective at inhibiting hepatic fatty acid synthesis activity.

LONGITUDINAL STUDY OF ADIPOSITY IN CHICKENS SELECTED FOR HIGH OR LOW ABDOMINAL FAT CONTENT: FURTHER EVIDENCE OF A GLUCOSE-INSULIN IMBALANCE IN THE FAT LINE. J. Simon and B. Leclercq (Station de Recherches Avicoles, Inst. Natl. de la Recherche Agronomique, 37380 Nouzilly, France) *J. Nutr. 112* (10): 1961-1973 (1982). Selected fat (FL) or lean (LL) lines of chickens have been further studied. Total lipid and abdominal fat content and size of adipocytes isolated from the gizzard were significantly increased in both sexes of the fat line from 2 to 4 weeks of age onwards. The divergence in abdominal fat content was maximum at 9 weeks of age. Both in the fed and the

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fasted state, the plasma glucose level was lower in FL than in LL chickens, at hatching and shortly after. This was not, however, associated with higher plasma insulin levels in FL chickens. At 2 weeks of age, insulin content of the pancreas did not differ. From 5-8 weeks of age after ad libitum refeeding or forced-feeding following at fast, plasma glucose increased to similar levels in both lines but in contrast, plasma insulin levels were largely enhanced in FL chickens. At 17 weeks of age, glucose clearance was faster in FL chickens and associated with a slightly (although nonsignificant) higher insulin release. In eggs laid by FL hens, yolk weight was disproportionately increased and albumen glucose content was decreased. During the last third of embryonic development, plasma glucose levels were similar in both lines at the F_4 generation and in contrast lower in the FL embryos at the F_5 generation. The physiological situation of FL chickens appears therefore very similar to short-lived preobese state observed in mammals.

EFFECT OF BRANCHED CHAIN VOLATILE FATTY ACIDS, TRYPTICASE, UREA, AND STARCH ON IN VITRO DRY MATTER DISAPPEARANCE OF SOYBEAN STOVER. R. Soofi, G.C. Fahey, Jr., L.L. Berger, and F.C. Hinds (Department of Animal Science, University of Illinois, Urbana, IL 61801) *J. Dairy Sci. 65* (9): 1748-1753 (1982). We measured in vtiro effects of branched chain fatty acids, Trypticase (a pancreatic digest of casein, a bacteriological peptone), urea, starch, and their interactions on dry matter disappearance of soybean stover. Quantities of nutrients added to fermentation media were equal to amounts of nutrients contributed by one part alfalfa added to two parts soybean stover. Maximum digestibilities were between 48 and 56 h except when branched chain fatty acids and Trypticase were added to the medium together. In this case, a linear increase in digestion occurred even at 72 h. There was a negative effect of starch and a positive effect of branched chain fatty acids on in vitro dry matter disappearance of soybean stover. Among the interactions, only the interaction between branched chain fatty acids and starch was significant, indicating either the negative effect of starch on utilization of branched chain fatty acids and starch by remen microorganisms at the expense of fiber. Branched chain fatty acids and Trypticase together appeared to provide a balanced medium for bacterial growth.

TRANSMETHYLATION OF PHOSPHATIDYLETHANOLAMINE: AN INITIAL EVENT IN EMBRYONIC CHICKEN LENS FIBER CELL DIFFERENTIATION. P.S. Zelenka, D.C. Beebe, and D.E. Feagans (Laboratory of Molecular and Developmental Biology, National Eye Institute of Health, Bethesda, Maryland 20205) *Science 217*: 1965-1967 (1982). Agents that induce differentiation of lens epithelial cells into lens fiber cells in vitro transiently stimulate the transmethylation of phosphatidylethanolamine. Inhibition of transmethylation by 3-deazaadenosine results in a corresponding inhibition of the cells elongation that characterizes lens fiber formation, suggesting that phospholipid methylation plays an essential role in the differentiation of these cells.

THE EFFECT OF CHOLESTYRAMINE AND MEVINOLIN ON THE DIURNAL CYCLE OF RAT HEPATIC 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE. R.D. Tanaka, P.A. Edwards, S-F. Lan, E.M. Knoppel, and A.M. Fogelman (Div. of Cardiology, Dept. of Med. and the Dept. of Biol. Chem., Univ. of California, Los Angeles, Los Angeles, CA 90024) *J. Lipid Res. 23* (7):1026-1031 (1982). Rats were fed powdered rat chow or a rat chow diet containing 5% cholestyramine or 5% cholestyramine with Mevinolin (112 mg/100 g food, 200 mg/kg body weight per day). The specific activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase was determined at different times during the diurnal cycle of the enzyme. Animals fed cholestyramine had higher specific activities of HMG-CoA reductase at all time points tested when compared to controls. The specific activity at the peak in the diurnal cycle was approximately 8-fold higher in cholestyramine-treated animals. Rats administered the cholestyramine-Mevinoline diet had higher specific activities of the enzyme than either cholestyramine-treated or control animals. In the cholestyramine-Mevinolin-treated animals the peak in the diurnal cycle was shifted to D-12 (12th hour of the dark cycle) and the specific activity at this point was approximately 133-fold greater than the basal (L-6) activity in control animals. Optimal conditions for immunotitration studies were determined such that valid conclusions could be drawn from these data. Based on immunotitration experiments, the increased hepatic HMG-CoA reductase activity in cholestyramine-treated animals resulted in part from a 3-fold activation of the enzyme, while the increased specific activity in the cholestyramine-Mevinolin-treated animals was due solely to increased enzyme mass.

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