

Abstracts

Biochemistry and nutrition

A FLUORESCENCE STUDY OF APOLIPOPROTEIN LOCALIZATION IN RELATION TO LIPIDS IN SERUM LOW DENSITY LIPOPROTEINS. G.E. Dobretsov, M.M. Spirin, O.V. Chekrygin, I.M. Karmansky, V.M. Dmitriev and Yu.A. Vladimirov (2nd Moscow State Med. College and Inst. of Biol. and Med. Chem., Academy of Med. Sci., 117437 Moscow (U.S.S.R.)) *Biochimica et Biophysica Acta* 710(2):172-180 (1982). Fluorescence energy transfer studies were carried out on low density lipoproteins (LDL) containing pyrene, in order to investigate their structure. The results indicate that almost all of the LDL tryptophan residues are located in the same surroundings near the surface of the particle and are immersed in the lipid phase 10-20 Å below the lipid/water interface. The data do not support a model of protein spikes protruding from the particle surface. Such spikes have been observed in LDL preparations only after long-term storage.

INTESTINAL ABSORPTION OF BILIARY AND EXOGENOUS CHOLESTEROL IN THE RAT. C. Dulery and D. Reisser. (Laboratoire de Physiologie animale et de Nutrition (LA 273 CNRS), UER Nutrition, BP 138, 21004 Dijon Cedex (France)) *Biochimica et Biophysica Acta* 710(2):164-171 (1982). Non-starved rats (fed a cholesterol-free diet prior to the experiments) with common bile fistula were infused intraduodenally with rat bile labelled with [1,2-³H] cholesterol at a constant rate (0.6 ml/h) and a nutritive mixture containing, in particular, olive oil and a 1 μmol [4-¹⁴C] cholesterol per ml at rates of 1 ml/h (group B) or 2.3 ml/h (group A) for 5 h. Control rats (group C) were prepared as group B rats but the nutritive mixture was free of cholesterol. 1 h after the end of infusions, the animals were killed. Biliary and exogenous cholesterol were absorbed in the upper two-thirds of the small intestine; a large proportion of ³H and ¹⁴C radioactivity was present in the mucosa, but cholesterol from exogenous origin went across the mucosa more rapidly than cholesterol from biliary source. These observations suggest the existence of a non-homogeneous luminal mixture of molecules of cholesterol from different sources. The luminal dilution of [³H]- and [¹⁴C]sterols by non-labelled sterols increased from the proximal to the distal part of the small intestine. Precursor sterols and coprosterol were present in the stomach contents and in the lumen of caecum, colon and feces.

DIETARY "MEATS" AND SERUM LIPIDS. M.A. Glynn, H.D. Naumann, G.B. Nolph, G. Krause, M. Ellersieck. (Dept. of Family and Community Med., Schl. of Med., Columbia, MO 65212) *Am. J. Clin. Nutr.* 35(5):935-942 (1982). The mean values for serum total cholesterol for 47 males, aged 32 to 62 yr. who, over a 10½ month study ate, within a self-selected diet, beef as the only meat for 3 months, poultry and fish for 3 months, and pork for 3 months showed no statistically significant difference, whereas 17 of 29 females of the same age who participated in the same study had borderline statistically significant differences in mean values ($p < 0.05$). When data from all subjects were considered together, no statistically significant changes in mean high-density lipoprotein cholesterol values, both upward and downward. The important results of this study are documentary to the lack of influence of "meat" and its fat on effecting a significant change in serum total cholesterol within a self-selected diet.

EFFECT OF ZINC SUPPLEMENTATION ON PLASMA HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AND ZINC. J.H. Freedom-Graves, B.J. Friedman, W. Han, R.L. Shorey, R. Young (Division of Graduate Nutr., GEA 115, Univ. of Texas at Austin, Austin, TX 78712) *Am. J. Clin. Nutr.*, 35:988-992 (1982). The recent report by Hooper PL, et al. (JAMA 1980;244:1960-1) that pharmacological doses (160 mg) of zinc lowered high-density lipoprotein (HDL)-cholesterol in men and that zinc might be an atherogenic agent prompted this report of the effect of zinc supplementation on HDL-cholesterol in women. Four levels of zinc supplements (0, 15, 50, or 100 mg/day) were given to 32 women for 8 wk. Fasting plasma HDL-cholesterol and zinc were measured at biweekly intervals. Plasma zinc increased in the supplemented groups, peaked at wk 4, then decreased toward initial values. The decline in plasma zinc regardless of continuing zinc administration may reflect a homeostatic response. No significant differences were seen in HDL-cholesterol over the 8 wk except in the 100 mg group at wk 4 when a transient decrease, -8.4% (57 to 48 mg/dl, $p < 0.04$) was observed. Thus we

conclude that in women the reduction in HDL-cholesterol in response to the pharmacological doses of zinc used in this study was transient and not dose-related.

STRUCTURE AND COMPOSITION OF HYDROCARBONS AND FATTY ACIDS FROM A MARINE BLUE-GREEN ALGA, *SYNECHOCOCCUS* SP. R.S. Goodloe, R.J. Light (Dept. of Chem., Florida State Univ., Tallahassee, FL 32306) *Biochim. Biophys. Acta* 710:485-492 (1982). The major hydrocarbons of *Synechococcus* sp., a marine blue-green alga isolated from the Gulf of Mexico, were identified as 1-nonadecene and 1,14-nonadecadiene. The content of 1-nonadecene increased with culture age from about 0.5 mg/g dry weight in young cells to about 2.3 mg/g dry weight in old cells, while the content of 1,14-nonadecadiene remained constant with culture age at about 1 mg/g dry weight. Both [1-¹⁴C]acetate and [2-¹⁴C]acetate were incorporated to equal extents into the hydrocarbons. [1-¹⁴C]Stearate was incorporated into the hydrocarbons, but [³H]arachidate was not. The fatty acids of *Synechococcus* sp. were typical of blue-green algae, consisting of C_{16:0}, C_{16:1}, C_{18:0}, C_{18:1}, C_{18:2}, and C_{18:3}. No C₂₀ fatty acids were detected. The hexadecenoic acid was shown to be 9-C_{16:1} while the octadecenoic acid was a mixture of 93% 11-C_{18:1} and 7% 9-C_{18:1}. The fatty acid content increased during the first 4 days of growth and then decreased slightly.

DECREASED PHOSPHOLIPASE A₂ ACTIVITY AND PROSTAGLANDIN BIOSYNTHESIS IN BACILLUS CALMETTE-GUERIN-ACTIVATED ALVEOLAR MACROPHAGES. W. Hsueh, R. Lamb, F. Gonzalez-Crussi (The Dept. of Pathology, The Children's Memorial Hospital, Northwestern Univ. Med. Schl., Chicago, IL 60614) *Biochim. Biophys. Acta* 710:406-414 (1982). Alveolar macrophages from normal and BCG-infected rabbits were labelled with [¹⁴C]arachidonate and its metabolites were measured. It was found that production of both prostaglandins and 12-hydroxyicosatetraenoic acid was diminished markedly in BCG-primed macrophages. This decrease was due partly to a depression in cyclooxygenase and lipoxygenase activity, but mainly to a decrease in arachidonic acid release, probably due to a suppression in phospholipase A₂ activity. This is indicated by a consistent depression of this enzyme activity in BCG macrophage homogenates at a wide pH range, suggesting that both lysosomal and non-lysosomal phospholipases are suppressed in BCG macrophages. However, intracellular lysosomal acid phosphatase and its release were increased markedly in BCG-primed macrophages. Our previous studies have shown a close relationship between lysosomal acid hydrolase release and production of arachidonate-prostaglandins in normal macrophages. The present study shows that in activated macrophages primed by BCG, different mechanisms are operative in the control of synthesis and release of lysosomal acid hydrolases and of the phospholipase for prostaglandin production.

GROWTH, HEART WEIGHT, CARDIAC LIPID, AND PATHOLOGY OF CHICKENS FED SOYBEAN OIL OR OIL EXTRACTED FROM DIFFERENT RAPESEED CULTIVARS. H.W. Hulan, A.H. Corner, D.M. Nash, and F.G. Proudfoot (Res. Centre, Agriculture Canada, Kentville, Nova Scotia B4N 1J5 and Animal Pathology Division, Animal Diseases Res. Inst., Agriculture Canada, Nepean, Ontario K2H 8P9) *Poultry Sci.* 61(6):1154-1166 (1982). A total of 384 male Single Comb White Leghorn chicks of the Hyline strain were randomized in 24 Petersime battery units of 16 birds per unit. Four replicate pens were fed either a basal (control) diet containing no added oil or a diet supplemented with 20% by weight of either soybean oil or rapeseed oil (RSO) from cultivars Tower, Candle, Regular, or R-500. Levels of erucic acid (22:1) in the RSO's ranged from .1 to 51.6%. Four birds from each unit were killed at 4, 8, 12, and 16 weeks for cardiac lipid analysis and pathology. Feeding RSO had no consistent effect on body weights, heart weights, or the heart-to-body weight ratio. Only the diet containing R-500 (51.6% 22:1) consistently increased cardiac lipid levels as measured gravimetrically. Maximum deposition of the long-chain monoenes (22:1) and eicosenoic acids (20:1) occurred in the cardiac lipids by 8 weeks, and the levels remained high throughout the 16-week trial. The relative concentration of these long-chain monoenes in the cardiac lipids was positively correlated to the dietary levels of these acids. The incidence of lesions found in heart, liver, and skeletal muscle was significantly higher in chickens fed RSO's high in 22:1 than in

chickens fed control or soybean oil, and the incidence significantly correlated to the level of 22:1 in the diet. The incidence of heart and muscle lesions and sinusoidal distention in chickens fed RSO's low in 22:1 was not significantly different from that of chickens fed the control diet or soybean oil, but the incidence of hepatic necrosis was significantly higher for chickens fed RSO's low in 22:1 compared to those fed the control diet or soybean oil.

ETHANOLAMINE PLASMOLOGEN & CHOLESTEROL ESTER METABOLISM IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS. H.M. Jagannatha and P.S. Sastry (Dept. of Biochem., Indian Inst. of Sci., Bangalore 560 012) *Indian J. Biochem. Biophys.* 18(6):411-416 (1981). Activities of fatty acid reductase, O-alkyl-dihydroxyacetonephosphate (DHAP) synthase, 1-O-alkenyl-Sn-glycerophosphoryl ethanolamine (GPE) 2-acyl transferase and cholesterol ester synthases (active at pH 5.2 and pH 7.4) have been determined in various regions of the central nervous system at the onset (Stage I), maximum paralysis (Stage II) and recovery state (Stage III) of the acute period of experimental allergic encephalomyelitis (EAE) induced in adult rats. At stage II, fatty acid reductase activity was decreased to 55% in whole brain and to 48% in cerebellum of that in adjuvant-treated controls. At this stage, O-alkyl HDAP synthase showed 54, 11 and 20% decrease in activity of whole brain, cerebellum and brain stem respectively; Activities of both the enzymes improved towards normal values at the recovery stage. O-alkenyl GPE acyl transferase was much less affected. The pH 5.2 cholesterol ester synthase increased to 132% of control only in spinal cord at stage II. The pH 7.4 cholesterol ester synthase increased over 240% in all stages in the cerebellum and also showed higher activity in brain stem and spinal cord in stage II. These results suggest that the decrease in the enzyme levels studied in EAE may be due to damage to glial cells and that the pH 7.4 cholesterol ester synthase is more likely to be responsible for cholesterol ester synthesis in demyelinating conditions.

TRIACYLGLYCEROL LIPASE, MONOACYLGLYCEROL LIPASE AND PHOSPHOLIPASE ACTIVITIES OF HIGHLY PURIFIED RAT HEPATIC LIPASE. G.L. Jensen, B. Daggy, A. Bensadoun (Division of Nutritional Sci., Cornell Univ., Ithaca, NY 14853) *Biochim. Biophys. Acta* 710:464-470 (1982). Highly purified rat hepatic lipase (NaCl-resistant, alkaline pH optimum) was studied to evaluate whether the enzyme has triacylglycerol lipase, monoacylglycerol lipase and phospholipase activities. Enzyme exhibiting a single band by SDS-polyacrylamide gel electrophoresis and having a specific activity eight times greater than that in any previous report was utilized. The ratios of the different lipolytic activities to each other remained constant throughout a multistep hepatic lipase purification. The lipolytic activities coeluted by gel filtration on Ultragel Aca 34. Column isoelectric focusing of the highly purified enzyme revealed comigration of the lipolytic activities. Thermal inactivation produced similar decay curves for the different activities. Immune titration curves for the different activities with specific antibody against hepatic lipase were essentially identical. These findings indicate that hepatic lipase is a single enzyme molecule which has triacylglycerol lipase, monoacylglycerol lipase and phospholipase activities with artificial substrates. To study these lipolytic activities further, highly purified hepatic lipase was subjected to limited digestion by specific proteases. The triacylglycerol lipase activity was more sensitive to proteolytic destruction than either of the other two activities.

EFFECT OF DIETARY LEVEL OF SULFUR-CONTAINING AMINO ACIDS ON LIVER DRUG-METABOLIZING ENZYMES, SERUM CHOLESTEROL AND URINARY ASCORBIC ACID IN RATS FED PCB. N. Kato, S. Mochizuki, K. Kawai, A. Yoshida (Dept. of Agr. Chem., Nagoya Univ., Nagoya 464, Japan) *J. Nutr.* 112:848-854 (1982). Effects of dietary level of sulfur-containing amino acids (S-AA) on liver drug-metabolizing enzymes, serum cholesterol and ascorbic acid metabolism in growing rats fed diets containing 300 ppm of polychlorinated biphenyls (PCB) were investigated. Maximum gain in body weight was observed with 0.5% S-AA diets with or without PCB addition. Metabolic parameters increased by PCB were liver weight, activities of hepatic aminopyrine N-demethylase and aniline hydroxylase, serum total cholesterol, serum high density lipoprotein cholesterol, serum corticosterone and urinary metabolites of the glucuronic acid pathway including ascorbic acid, glucuronic acid and glucaric acid. In the PCB-treated animals, maximum values of liver weight, aminopyrine demethylase activity, serum cholesterol, serum corticosterone, urinary ascorbic acid and glucaric acid were obtained with about 0.8% S-AA. For the maximum induction of these metabolic responses, 0.5% S-AA was not enough. Urinary glucuronic acid and the ratio of lower density lipoprotein cholesterol versus high density lipoprotein cholesterol were decreased with a supplement of S-AA to PCB-containing diets.

METABOLISM OF PLASMA MEVALONATE IN RATS AND HUMANS. R.R. Kopito, S.B. Weinstock, L.E. Freed, D.M. Murray, H.

Brunengraber (Dept. of Nutr. and Food Sci., Massachusetts Inst. of Tech., Cambridge, MA 02139) *J. Lipid Res.* 23(4):577-583 (1982). A circadian rhythm in plasma mevalonate was identified in human subjects. This variation, over a 5-fold range, is paralleled by a rhythm in urinary excretion. No such diurnal change in plasma mevalonate was observed in schedule-fed, light-cycled rats, despite the presence of a pronounced rhythm in liver HMG-CoA reductase and sterol synthesis. A linear correlation was found between liver HMG-CoA reductase activity and the rate of hepatic sterol synthesis. Sterol synthesis accounted for 59% of the HMG-CoA reductase activity. A 4-fold increase in plasma mevalonate following bilateral nephrectomy did not feed back on liver HMG-CoA reductase. Turnover rates for circulating R- and S-mevalonate were determined by the kinetics of tritiated tracers. S-Mevalonate exhibited first-order kinetics with a $T_{1/2}$ of 19 to 23 min, while R-mevalonate kinetics could be resolved into two phases with half-lives of 9 and 42 min. The renal uptake of circulating mevalonate was measured by the initial rate of increase in plasma mevalonate immediately following bilateral nephrectomy; this was confirmed by determination of the renal arterio-venous difference. This value ranges between 500 and 600 pmol/min for a 250-g rat.

INFLUENCE OF NATIVE AND RANDOMIZED PEANUT OIL ON LIPID METABOLISM AND AORTIC SUDANOPHILIA IN THE VERVET MONKEY. D. Kritchevsky, L.M. Kavidson, M. Weight, N.P.J. Kriek, J.P. du Plessis (The Wistar Inst. of Anatomy and Biol., 36th Str. at Spruce, Philadelphia, PA 19104) *Atherosclerosis* 42:53-58 (1982). Vervet monkeys (*Cercopithecus aethiops pygmaeus*) were fed cholesterol-free, semipurified diets containing 40% sucrose, 25% casein, 15% cellulose and 14% peanut oil (PNO), randomized peanut oil (RPNO) or corn oil (CO). After 4 months, serum cholesterol and triglyceride levels, serum lecithin-cholesterol acyl transferase (LCAT) activity and plasma lipoprotein lipase (LPL) activity were similar in all groups. Livers of monkeys fed CO converted 156% more acetate and 24% more mevalonate to cholesterol than those of monkeys fed RPNO. Cholesterogenesis in RPNO-fed monkeys was enhanced compared to PNO (68% from acetate; 62% from mevalonate). Incidence of atherosclerosis was 33% in monkeys fed RPNO, 80% in those fed CO and 90% in those fed PNO. Extent of sudanophilia was lowest in aortas of monkeys fed RPNO. Incidence of arteriosclerosis was 40% in monkeys fed CO, 56% in those fed RPNO and 70% in those fed PNO. Extent of aortic surface showing arteriosclerosis was highest in monkeys fed RPNO.

THE EFFECT OF ANESTHESIA OR RESTRAINT ON TRIACYLGLYCEROL TURNOVER IN THE RAT. B.R. Krause, L. Dory, P.S. Roheim (Dept. of Physiology, Louisiana State Univ. Med. Center, New Orleans, LA 70112) *Biochim. Biophys. Acta* 710:471-476 (1982). A comparison of triacylglycerol metabolism was made among anesthetized, restrained and unanesthetized-unrestrained rats. The method utilized for the comparison was the determination of triacylglycerol turnover following the intravenous injection of [3 H]glycerol. Peak appearance of triacyl[3 H]glycerol was 25 min in unanesthetized-unrestrained rats but 35 and 45 min in restrained and anesthetized rats, respectively. Using serial plasma triacylglycerol determinations as an index of steady-state in all three groups, it was found that only the anesthetized and unanesthetized-unrestrained animals could be used for kinetic analysis. In these two animal preparations, apparent fractional catabolic rates were calculated and found to be lower in anesthetized (0.014 min^{-1}) compared to unanesthetized-unrestrained animals with chronic indwelling cannulas (0.029 min^{-1}). Apparent total catabolic rates, calculated from the plasma triacylglycerol mass and estimated plasma volume, were reduced by 50% in anesthetized animals. It is concluded that wide differences in triacylglycerol metabolism exist in these rat preparations which should be considered in the interpretation of future studies. The unanesthetized-unrestrained rat model may represent the closest approximation to the normal physiologic state.

A CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDY OF AORTIC LESIONS AND CHOLESTERYL ESTER RICH LIPOPROTEINS FROM ATHEROSCLEROTIC RABBITS. P. Kroon, D. Quinn, E. Cordes (Merck Inst. for Therapeutic Res. Merck Sharp & Dohme Res. Labs., Rahway, NJ 07065) *Biochem.* 21(1):2745-2753 (1982). Rabbits on a diet supplemented with cholesterol had increased plasma cholesterol levels and developed atherosclerosis. Most of the plasma cholesterol exists as cholesteryl esters in very low density and low-density lipoproteins. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra (at 48°C) of lipoproteins, and of arterial lesions from cholesterol-fed rabbits, are dominated by well-resolved cholesteryl ester resonances. An analysis of line widths shows that the cholesteryl esters are in a liquid state at this temperature. Based on line widths and spin-lattice relaxation times, the motion of the cholesteryl ester molecules is highly anisotropic; motion about the long axis of the cholesteryl moiety is 38-75 times faster than motion about the short axis. Spectra for the lipoproteins and arterial lesions show temperature-dependent line-width changes that

are consistent with an order-disorder transition of the cholesteryl esters above physiological temperatures. The similarity of line widths and spin-lattice relaxation times for lipoproteins and arterial lesions indicates that their molecular organization and molecular dynamics are also similar and suggests that an appreciable fraction of the cholesteryl esters are derived from nonmetabolized lipoproteins. Only phospholipid choline methyl resonance is different in lipoproteins and lesions. The lipoprotein choline methyl resonance is relatively narrow at all temperatures studied, consistent with a fluid phospholipid monolayer. The same resonance for arterial lesions is 2.5 times broader. The increased line width is due in part to a more heterogeneous environment in the arterial lesions.

PLASMA LIPIDS AND LIPOPROTEINS IN JAPANESE MALE PATIENTS WITH CORONARY ARTERY DISEASE AND IN THEIR RELATIVES. H. Kukita, Y. Imamura, M. Hamada, T. Joh, and T. Kokubu (Second Dept. of Internal Med., Ehime Univ., School of Med., Onsen-gun, Ehime 791-02) *Atherosclerosis* 42(1)21-29 (1982). Plasma cholesterol (CH), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) were measured in 92 consecutive Japanese male subjects undergoing diagnostic coronary cineangiography. Sixty-nine of them were classified as having coronary artery disease (CAD), the remaining 23 subjects were classified as having normal coronary arteries (NCA). The CAD group has significantly lower HDL-C and higher TG levels than the NCA group. However, there was no significant difference in plasma CH between the two groups. First-degree relatives of the CAD patients were also investigated. The male blood relatives of the CAD patients also had significantly lower HDL-C and higher TG levels than the non-blood male relatives and healthy control males. The female blood relatives, however, showed no significant differences from the non-blood female relatives and the healthy control females in plasma CH, TC and HDL-C levels. These results suggest that low HDL-C and hypertriglyceridemia are the prevalent coronary risk factors, rather than hypercholesterolemia, in a population with a low fat intake such as the Japanese, and that these lipid abnormalities are related to sex and genetic factors.

CALMODULIN-MEDIATED REGULATION OF CALCIUM TRANSPORT AND ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-ACTIVATED ATPASE ACTIVITY IN ISOLATED CARDIAC SARCOPLASMIC RETICULUM. M.A. Kirchberger and T. Antonetz (Dept. of Physiology and Biophys., The Mount Sinai Schl. of Med. of the City Univ. of New York, NY 10029) *J. Biol. Chem.* 257(10):5685-5691 (1982). A severalfold activation of calcium transport and ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-activated ATPase activity by micromolar concentrations of calmodulin was observed in sarcoplasmic reticulum vesicles obtained from canine ventricles. This activation was seen in the presence of 120 mM KCl. The ratio of moles of calcium transported per mol of ATP hydrolyzed remained at about 0.75 when calcium transport and ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-activated ATPase activity were measured in the presence and absence of calmodulin. Thus, the efficiency of the calcium transport process did not change. Stimulation of calcium transport by calmodulin involves the phosphorylation of one or more proteins. The major ^{32}P -labeled protein, as determined by sodium dodecyl sulfate slab gel electrophoresis, was the 22,000-dalton protein called phospholamban. The Ca^{2+} concentration dependency of calmodulin-stimulated microsomal phosphorylation corresponded to that of calmodulin-stimulated ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-activated ATPase activity. Proteins of 11,000 and 6,000 daltons and other proteins were labeled to a lesser extent. A similar phosphorylation pattern was obtained when microsomes were incubated with cAMP-dependent protein kinase and ethylene glycol bis (β -aminoethyl ether)-N,N,N',N'-tetraacetic acid. Phosphorylation produced by added cAMP-dependent protein kinase and calmodulin was additive. These studies provide further evidence for Ca^{2+} -dependent regulation of calcium transport by calmodulin in sarcoplasmic reticulum that could play a role in the beat-to-beat regulation of cardiac relaxation in the intact heart.

LIPOTROPIC EFFECT OF ESTRADIOL-17 β IN FED AND FASTED LACTATING COWS. B. Laarveld, B. DeLorme, and D.E. Kerr (Dept. of Anim. Sci., Macdonald College of McGill Univ., Ste. Anne de Bellevue, Quebec, Canada H9X 1C0) *J. Dairy Sci.* 65(6):920-926 (1982). The lipotropic effect of estradiol-17 β was measured in seven nonpregnant Holstein-Friesian cows 2 to 5 weeks into lactation. The experiment was divided into an initial 3-day control period, 19 days treatment, and 2 days fast. Jugular blood samples were taken twice daily during the control period, the last 2 days of treatment and the fast. After the control period, four cows received daily injections of estradiol-17 β benzoate (15 mg) in sesame oil for the remainder of the experiment whereas three cows received injections of sesame oil only. Estradiol-17 β did not change triglyceride concentration of plasma during the treatment period but increased it markedly during fast. Estradiol-17 β increased the free glycerol concentration of plasma during treatment. Cholesterol concentrations of plasma showed a consistent trend lower in the estradiol group during treatment and fast. No differences were apparent in glucose and blood ketone con-

centrations. The concentration of insulin in plasma was lower for the estradiol group during treatment. Estradiol-17 β appears to have a similar effect on lipid metabolism in dairy cows to other species. However, it exerted a strong lipotropic effect only under fasting.

INFLUENCE OF DIETARY SEX HORMONES ON CHICK LIPID METABOLISM. D.E. Leszczynski, T. Toda, and F.A. Kummerow (Harlan E. Moore Heart Research Foundation, Illinois and Burnside Research Laboratory, Univ. of Illinois, Urbana, IL 61801) *Horm. Metab. Res.* 14(4):183-189 (1982). The major ovarian hormones (estradiol and progesterone), the major testicular hormone (testosterone), and the major precursor to steroid hormones (cholesterol) were fed ad libitum in various combinations for either 2 weeks or 2 months (56 days) to 5-day-old female chicks; the effects of these treatments on liver lipids and plasma lipoproteins were measured. After two weeks, chicks fed basal diet supplemented with 0.05% estradiol had significant increases in plasma total cholesterol, triglycerides, and phospholipids ($P < .01$). The combined supplementation of 0.05% estradiol plus 1% cholesterol produced an additive increase in plasma total cholesterol resulting in levels higher than obtained by either treatment alone ($P < .01$). The addition of 0.2% progesterone to the 1% cholesterol diet inhibited the accumulation of plasma cholesterol ($P < .05$), liver cholesterol ($P < .01$), and liver triglycerides ($P < .01$), which were found in comparable animals fed only 1% cholesterol. Likewise, the addition of 0.2% progesterone to diet containing 0.05% estradiol inhibited ($P < .01$) accumulations in all of the plasma lipid classes which were found in comparable birds fed only 0.05% estradiol. After two months feeding, 0.1% testosterone had no effect on plasma or liver lipids. The combination of 0.01% estradiol plus 0.1% testosterone for 2 months was not very effective in reducing the hyperlipidemia caused by estradiol alone, but this treatment did result in a peculiar dwarf chicken. The results demonstrate strong steroid sex hormone interactions which produce major changes in chick plasma and liver lipid metabolism.

ALTERATIONS OF THE γ -CARBOXYGLUTAMIC ACID AND OSTEOCALCIN CONCENTRATIONS IN VITAMIN D-DEFICIENT CHICK BONE. J.B. Lian, M.J. Glimcher, A.H. Roufosse, P.V. Hauschka, P.M. Gallop, L. Cohen-Solal, B. Reit (Dept. of Biol. Chem. and Oral Biol., Harvard Med. Schl. and Harvard Schl. of Dental Med., Boston, MA 02115) *J. Biol. Chem.* 257(9):4999-5003 (1982). The content of osteocalcin and protein bound γ -carboxyglutamic acid (Gla) was studied as a function of bone maturation and mineralization in normal and vitamin D-deficient, rachitic chickens. The Gla/ Ca^{2+} ratio was elevated in rachitic bone, particularly in the most undermineralized regions. For example, there is a 10- to 20-fold elevation in Gla/ Ca^{2+} in the newly synthesized, least mineralized rachitic bone fraction, which progressively decreases to a 1.5-fold elevation in the most highly mineralized areas of rachitic tissue. Osteocalcin, which is the principal Gla-containing protein of mature bone, was quantitated by radioimmunoassay using specific antiserum to the 5670-dalton chicken protein. Surprisingly, the osteocalcin concentration is decreased 50% in vitamin D-deficient bone. From this we infer that accumulated Gla-containing protein in vitamin D-deficient and poorly mineralized bone may possibly represent a precursor of osteocalcin.

VITAMIN D₃ TOXICITY IN DAIRY COWS. E.T. Littledike and R.L. Horst (Natl. Animal Disease Center, Agric. Res., Sci. and Education Admin., US Dept. of Agric., PO Box 70, Ames, IA 50010) *J. Dairy Sci.* 65(5):749-759 (1982). Large parenteral doses of vitamin D₃ (15 to 17.5 $\times 10^6$ IU vitamin D₃) were associated with prolonged hypercalcemia, hyperphosphatemia, and large increases of vitamin D₃ and its metabolites in the blood plasma of nonlactating nonpregnant and pregnant Jersey cows. Calcium concentrations 1 day postpartum were higher in cows treated with vitamin D₃ about 32 days prepartum (8.8 mg/100 ml) than in control cows (5.5 mg/100 ml). None of the cows treated with vitamin D₃ showed signs of mild fever during the peripartur period; however, 22% of the control cows developed clinical signs of milk fever during this period. Signs of vitamin D₃ toxicity were not observed in nonlactating nonpregnant cows; however, pregnant cows commonly developed severe signs of vitamin D₃ toxicity and 10 of 17 cows died. There was a widespread metastatic calcification in the cows that died. Because of the extreme toxicity of vitamin D₃ in pregnant Jersey cows and the low margin of safety between doses of vitamin D₃ that prevent milk fever and doses that induce milk fever, we concluded that vitamin D₃ cannot be used practically to prevent milk fever when injected several weeks prepartum.

PROPERTIES AND METABOLIC FATE OF TWO VERY LOW DENSITY LIPOPROTEIN SUBFRACTIONS FROM RHESUS MONKEY SERUM. L. Lusk, J. Chung and A.M. Scanu (Depts. of Med. and Biochem., The Univ. of Chicago, Pritzker Schl. of Med., Chicago, IL 60637) *Biochimica et Biophysica Acta* 710(2):134-142 (1982). Physical, chemical and physiological approaches were used to examine the properties of two very low density lipoproteins,

VLDL-I and VLDL-II, which were isolated by agarose column chromatography from the serum of rhesus monkeys fed either Purina Chow or one of four hyperlipidemic diets containing 0.5-20% cholesterol suspended in either coconut oil, peanut oil, mixed coconut oil and butter fat or lard. In the coconut oil-fed hyperlipidemic animals, the majority of the apolar lipids of VLDL-I was represented by cholesteryl esters. The small percentage of triacylglycerol (15%) had a fatty acid composition which resembled that of the fatty acid in each of the diets. In turn, VLDL-II had a triacylglycerol-rich core and differed from VLDL-I in apolipoprotein distribution. Both VLDLs were hydrolyzed in vitro by milk lipoprotein lipase by first-order kinetics although VLDL-I exhibited a slightly slower reaction rate. When an oral dose of [³H] retinol was given to one of the animals, both VLDLs became labeled but the specific activity of VLDL-I was six times higher than that of VLDL-II and the other lipoproteins. We conclude that VLDL-I represents a cholesteryl ester-rich lipoprotein probably of intestinal origin, whereas VLDL-II may be a particle of hepatic derivation modified by its interaction with other plasma lipoproteins.

SPECTRAL CHARACTER OF SUNLIGHT MODULATES PHOTOSYNTHESIS OF PREVITAMIN D₃ AND ITS PHOTOISOMERS IN HUMAN SKIN. J.A. MacLaughlin, R.R. Anderson, M.F. Holick (Vitamin D Lab., Endocrine Unit, Mass. Gen. Hosp., Boston, MA 02114) *Science* 216(4549):1001-1003 (1982). The photosynthesis of previtamin D₃ from 7-dehydrocholesterol in human skin was determined after exposure to narrow-band radiation or simulation solar radiation. The optimum wavelengths for the production of previtamin D₃ were determined to be between 295 and 300 nanometers. When human skin was exposed to 295-nanometer radiation, up to 65 percent of the original 7-dehydrocholesterol content was converted to previtamin D₃. In comparison, when adjacent skin was exposed to simulated solar radiation, the maximum formation of previtamin D₃ was about 20 percent. Major differences in the formation of lumisterol₃ and tachysterol₃ from previtamin D₃ were also observed. It is concluded that the spectral character of natural sunlight has a profound effect on the photochemistry of 7-dehydrocholesterol in human skin.

THE EFFECTS OF FEED INTAKE ON ADIPOCYTES IN THE ABDOMINAL FAT PAD OF MATURE BROILER-TYPE FEMALE CHICKENS. B.E. March, S. Chu, C. Macmillan (Dept. of Poultry Sci., Univ. of British Columbia, Vancouver, Canada V6T 2A2) *Poultry Science* 61(6):1137-1146 (1982). One thousand broiler-type pullets were reared with feed restriction from 5 weeks of age to attain a target average body weight of 1.64 kg at 18 weeks of age. Thereafter feed was supplied at the rate of 130 g per bird per day until 47 weeks at which time half the population was fed *ad libitum*. An additional treatment was imposed at 86 weeks when 12 birds were gradually but severely restricted in feed intake until, by 97 weeks, they were receiving only 40 g feed per day. Birds restricted in feed intake until 47 weeks and then fed *ad libitum* increased consumption sharply and in proportion to body weight for approximately 2 weeks. Thereafter, intake declined to about 20% in excess of the restricted level for the next 16 weeks. Body weight increased with the increased feed intake. Average adipocyte diameter increased with the age of the birds even when feed was restricted. There was no increase in the number of adipocytes present in the retroperitoneal fat pad after 27 weeks of age. The size distribution of adipocytes was consistently bimodal except following severe feed restriction when the weight of the fat pad regressed and the secondary peak of small adipocytes disappeared.

THE ROLE OF ENDOGENOUS PHOSPHATIDYLCHOLINE AND CERAMIDE IN THE BIOSYNTHESIS OF SPHINGOMYELIN IN MOUSE FIBROBLASTS. W.D. Marggraf, R. Zertani, F.A. Anderer, J.N. Kanfer (Friedrich Miescher Labor der Max-Planck Gesellschaft, Spemannstr. 37-39, 7400 Tübingen, F.R.G.) *Biochim. Biophys. Acta* 710:314-323 (1982). The intracellular location of sphingomyelin formation via the cholinephosphotransferase reaction from both endogenous and exogenous phosphatidylcholine and ceramide substrates has been studied in the subcellular membrane fractions prepared from mouse fibroblasts. The enzyme was found to be located in both the plasma membrane and the Golgi fractions. Activity in the Golgi fraction was stimulated to a greater extent by the addition of exogenous ceramide than was the activity in the plasma membrane fraction. It is concluded that endogenous phosphatidylcholine is available to the cholinephosphotransferase at saturating concentration, therefore, is not rate-limiting. In contrast, the very low concentration of endogenous ceramide seems to limit the reaction rate, necessitating supplementation with exogenous material. Both endogenous substrates are shown to be utilized in an intramembranous rather than an intermembranous reaction. The capacity of the plasma membrane fraction to synthesize sphingomyelin from endogenous phosphatidylcholine and ceramide was found to be sufficiently high to account for the rate of net synthesis of plasma membrane-bound sphingomyelin observed in the logarithmically multiplying

cell culture. In contrast, the Golgi fraction displayed only 26% of the expected capacity, but it was stimulated 6-fold by the addition of exogenous ceramide. These results demonstrate that the total cellular sphingomyelin of the mouse fibroblasts can be provided via the cholinephosphotransferase pathway and that the plasma membrane and the Golgi fraction are most probably the intracellular sites of sphingomyelin biosynthesis.

PHYSICAL FITNESS AND PLASMA HDL CHOLESTEROL CONCENTRATIONS IN MALE BUSINESS EXECUTIVES. J.R.L. Masarei, J.E. Pyke, F.S. Pyke (Dept. of Clinical Biochem. and Human Movement and Recreation Studies, Univ. of Western Australia, Perth WA, Australia) *Atherosclerosis*, 42:77-83 (1982). Endurance fitness has been measured objectively (physical work capacity at pulse rate of 170/min, W₁₇₀) in a group of middle-aged executives, and related to a number of other physical characteristics and aspects of coronary risk status: FEV₁, blood pressure, adiposity, smoking habit, alcohol consumption, plasma levels of total and non-high density lipoprotein cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-C). The primary question was whether HDL-C levels could be shown to be related to endurance fitness levels over the range encountered in a fairly homogeneous population and hence whether there could be value in terms of lipid coronary risk status in encouraging a moderate increase in physical activity. HDL-C levels were significantly related to W₁₇₀. Fitness also separated the subjects in terms of adiposity, but not in terms of the other variables studied. Even though the trend was toward an index of physical activity being able to separate the subjects in terms of HDL-C, this was not as clear-cut as the division in terms of endurance fitness. Alcohol and smoking were associated with higher triglyceride levels, but not with HDL-C. The variables mid-abdominal skinfold thickness, triglyceride, non-HDL-C and endurance fitness accounted for 53% of the variation in HDL-C levels in this population. Alterations in the levels of these probably related variables might be expected to have appreciable effects on levels of HDL-C.

AN ULTRASTRUCTURAL AND STEREOLOGICAL STUDY ON LIPID DROPLETS OF ADRENAL CORTEX OF RATS FED WITH HIGH-FAT, LOW-PROTEIN AND CHOLESTEROL SUPPLEMENTS. H. Matsukuma (Second Dept. of Pathology, Nagasaki Univ. School of Med., Nagasaki, Japan) *Acta Med. Nagasaki* 26:56-72 (1982). In order to know the morphological change of the early phase of lipid formation in adrenocortical cells, the adrenal cortex of rats which were fed with high-fat, low-protein and cholesterol supplements was ultrastructurally observed with stereological analysis of lipid droplets in each zone. The amount and spherical size distribution of lipid droplets of the adrenal cortex were stable within 24 hours of the experiment contrasting to the liver of the same experimental rats which was previously observed that lipid droplets accumulated in the hepatocytes on and after 6 hours of experiment. This fact suggests the possibility that the amount of adrenocortical lipid droplets is stable under the hepatic regulation of cholesterol metabolism. And, also, each zone of the adrenal cortex was characterized by the amount and spherical size distribution of lipid droplets.

TEMPORAL ASSOCIATION BETWEEN ARTERIAL CHOLESTEROL DEPOSITION, THYMIDINE INCORPORATION INTO DNA, AND ATHEROSCLEROSIS IN JAPANESE QUAIL FED AN ATHEROGENIC DIET. D.L. McCormick, J.D. Radcliffe, R.G. Mehta, C.A. Thompson and R.C. Moon (Lab. of Pathophysiology, Life Sci. Div. IIT Res. Inst., Chicago, IL 60616) *Atherosclerosis* 42(1):1-13 (1982). Male Japanese quail (strain SEA) rapidly develop atherosclerotic lesions in the aorta and brachiocephalic arteries when fed an atherogenic diet containing 1.0% cholesterol and 0.5% cholic acid. The present study was conducted to determine time parameters of the atherosclerotic response. Groups of 20 quail fed the atherogenic diet were killed at 0 days, 1 day, 3 days, or weekly from 1 to 12 weeks. Quail fed the atherogenic diet for 1 day showed a significant increase in serum cholesterol a plateau was reached by 2 weeks. A significant increase in arterial cholesterol was seen after 2 weeks on the atherogenic diet, and arterial cholesterol showed a linear increase with time from 2 to 12 weeks. Increased incorporation of tritiated thymidine into the DNA of arterial cells was first seen at 2 weeks; thymidine incorporation increased to a maximum value at 9 weeks, then declined to 50-60% of the 9-week value at weeks 11 and 12. Grossly visible atherosclerotic lesions were first seen at 3 weeks, and 90% of birds showed gross atherosclerotic lesions by 8 weeks. Atherosclerosis induced in Japanese quail by feeding cholesterol and cholic acid is characterized initially by lipid deposition in the arterial wall, followed by increased incorporation of tritiated thymidine and the appearance of gross lesions.

THE BIOLOGICAL ORIGIN OF KETOTIC DICARBOXYLIC ACIDURIA. B. Mortensen, N. Gregersen (Res. Lab. for Metabolic Disorders, Univ. Dept. of Clinical Chemistry, Aarhus Kommunehospital, DK-8000 Aarhus C, Denmark) *Biochim. Biophys. Acta*

710:477-484 (1982). The β -oxidation of C_8 - C_{16} -dicarboxylic acids to short-chain dicarboxylic acids was investigated in vivo and in rat liver homogenate. The β -oxidation in vivo was evaluated from the excretions of C_6 - C_{10} -dicarboxylic acids in urine from rats given C_8 - C_{16} -dicarboxylic acids. Correspondingly, the β -oxidation in vitro was determined from the rise in concentration of C_6 - C_{10} ($_{12}$)-dicarboxylic acids in the postnuclear (600 X g) fraction of rat liver homogenates incubated with C_8 - C_{16} -dicarboxylic acids. The results showed that C_{10} - C_{14} -dicarboxylic acids were far better substrates for β -oxidation than were C_8 - and C_{16} -dicarboxylic acids. In particular, hexadecanedioic acid could only be β -oxidized to a minor degree, and, in contrast to the other dicarboxylic acids, it was toxic for starved rats. The activity of the lipid metabolism (unstarved, starved and diabetic ketotic rats) was of decisive significance for the quantity and pattern of the C_6 - C_{10} dicarboxylic acids present both in vivo and in vitro, since adipic acid was increased and sebatic acid decreased with increasing lipid catabolism, i.e. the adipic:sebatic acid ratio increased with increasing rates of β -oxidation. On comparison with earlier investigations on the chain-length dependency of the ω -oxidation of monocarboxylic acids it was concluded that the biological origin of the ketotic C_6 - C_8 -dicarboxylic aciduria is C_{10} - C_{14} -monocarboxylic acids, and that an elevated β -oxidation rate is important for the formation of C_6 - C_8 -dicarboxylic aciduria.

EFFECT OF PROBUCOL ON REPRODUCTIVE PERFORMANCE, EGG YOLK CHOLESTEROL CONTENT, AND LIPID METABOLISM IN THE LAYING HEN. E.C. Naber, J.F. Elliot, T.L. Smith (Dept. of Poultry Sci., Ohio State Univ., and Ohio Agr. Res. and Development Center, Columbus, OH 43210) *Poultry Science* 61(6): 1118-1124 (1982). Egg production type chickens were given the drug Probucol in their diets to determine its effect on egg production characteristics, liver lipid metabolism, egg yolk lipid synthesis, and egg yolk cholesterol concentration. In none of the three trials conducted did Probucol feeding affect egg production or body weight. The drug reduced total liver lipogenesis as measured by incorporation of ^{14}C -acetate by surviving liver slices. Relative incorporation of ^{14}C -acetate into cholesterol by liver was increased by the drug. However, *in ovo* incorporation of ^{14}C -acetate in total yolk lipids remains unchanged and relative incorporation into yolk cholesterol is reduced. As a result, egg yolk cholesterol content is reduced by 5% with the .10% dietary level of drug administration.

ISOLATION AND CHEMICAL CHARACTERIZATION OF TWO NEW VITAMIN D METABOLITES PRODUCED BY THE INTESTINE. N. Ohnuma, J.R. Kruse, G. Popják, A.W. Norman (Dept. of Biochem., Univ. of California, Riverside, CA 92521) *J. Biol. Chem.* 257(9):5097-5102 (1982). Two new vitamin D metabolites were isolated in pure form from incubations of 53 nM, 1,25-dihydroxyvitamin D_3 with homogenates of small intestinal mucosa of vitamin D-replete chicks. The birds were injected intravenously with 8 to 9 nmol of 1,25-dihydroxyvitamin D_3 /100 g body weight 5 to 8 h before death. The isolation involved methanol-chloroform extraction and four successive chromatographic procedures (Sephadex LH-20 and high performance liquid chromatography). Chemical structures of the metabolites are proposed on the basis of (a) their chromatographic behavior, (b) their mass spectra, and (c) ultraviolet absorption spectra. They are identified as 1 α -25-dihydroxy-23-oxo-vitamin D_3 and 1 α ,25,26-trihydroxy-23-oxo-vitamin D_3 . Neither of the two new metabolites is produced by the intestinal mucosa when 1,25S, 26-trihydroxyvitamin D_3 is used as a substrate.

UTILIZATION OF LONG-CHAIN FREE FATTY ACIDS IN WHITE AND RED MUSCLE OF RATS. G. Okano and T. Shimajo (Dept. of Physical Education and Dept. of Biochem., Sapporo Med. College, Sapporo 060 (Japan)) *Biochimica et Biophysica Acta* 710(2):122-127 (1982). Utilization of long-chain fatty acids was studied in white and red muscle slices prepared from quadriceps femoris muscle of rats, using radioactive palmitate and linoleate as precursors. After 2 h incubation, red muscle oxidized more palmitate to CO_2 and converted more of it to triacylglycerol and phospholipid compared with white muscle. The metabolic fate of palmitate incorporated into skeletal muscle fibers varied with muscle fiber types. In white muscle, 22% of incorporated palmitate was oxidized and 34% was converted to triacylglycerol and 43% to phospholipid. The percent distribution of radioactivity among CO_2 , triacylglycerol and phospholipid in red muscle was 44%, 22% and 34%, respectively. Percent distribution of the radioactivity in phospholipids was similar in white and red muscle. Predominant labeling with radioactive palmitate was found in phosphatidylcholine. Incubations with linoleate decreased the rates of oxidation and esterification of the fatty acid in both types of muscle compared with those with palmitate. The labeling profiles of radioactivity in muscle phospholipids with radioactive linoleate differed from those with palmitate. More linoleate was incorporated into phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, cardiolipin and phosphatidylglycerol compared with palmitate. Palmitate had faster rates of incorporation into phosphatidylcholine and sphingomyelin than linoleate. This

study showed that (a) utilization of fatty acids depended on muscle fiber types and (b) each fatty acid was oxidized to CO_2 and esterified to the lipid esters to a different extent by skeletal muscles.

4-BROMOCROTONIC ACID, AN EFFECTIVE INHIBITOR OF FATTY ACID OXIDATION AND KETONE BODY DEGRADATION IN RAT HEART MITOCHONDRIA. Y. Olowe, H. Schulz (Dept. of Chem., City College of the City Univ. of New York, New York, NY 10031) *J. Biol. Chem.* 257(10):5408-5413 (1982). 4-Bromocrotonic acid was found to effectively inhibit respiration supported by either palmitoylcarnitine or acetoacetate in coupled rat heart mitochondria. Partial inhibition was observed when 3-hydroxybutyrate served as a substrate, whereas pyruvate-supported respiration was unaffected by the inhibitor. Thus, 4-bromocrotonic acid inhibits fatty acid oxidation and ketone body degradation. When the enzymes of β -oxidation and ketone body degradation were assayed in mitochondria preincubated with 4-bromocrotonic acid, only 3-ketoacyl-CoA thiolase and acetoacetyl-CoA thiolase were found to be inactive. Evidence is presented for the enzymatic conversion of 4-bromocrotonic acid to 3-keto-4-bromobutryl-CoA which effectively inhibits both thiolases. A kinetic evaluation of the inhibitions caused by 4-bromocrotonic acid in coupled rat heart mitochondria demonstrated that 3-ketoacyl-CoA thiolase and respiration supported by palmitoyl carnitine are inactivated at equal rates. However, acetoacetyl-CoA thiolase was inactivated more rapidly than was respiration supported by acetoacetate. It is suggested that the thiolase-catalyzed step is rate-limiting in β -oxidation or is as slow as other reactions are. In contrast the thiolytic cleavage of acetoacetyl-CoA does not seem to be rate-limiting in ketone body degradation.

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THE CONCENTRATION OF APOLIPOPROTEIN A-I IN HUMAN PERIPHERAL LYMPH. D. Reichl, J.J. Pflug (Med. Res. Council Lipid Metabolism Unit, Hammersmith Hospital and Dept. of Surgery, Royal Postgraduate Med. Schl., Ducane Road, London W12 OHS, U.K.) *Biochim. Biophys. Acta* 710:456-463 (1982). The concentration of apolipoprotein A-I in peripheral lymph of eight apparently healthy subjects has been determined by quantitative immunoelectrophoresis. Under steady-state conditions the average concentration of the apolipoprotein in lymph was 15.9 ± 3.6 mg/dl, that is $12.24 \pm 2.3\%$ of its concentration in plasma of the corresponding subjects. Apolipoprotein A-I could not be detected immunochemically in particles smaller than haemoglobin ($M_r 67000$) when lymph was subjected to gel filtration on Sephacryl S300 superfine either by thin-layer or column gel filtration and apolipoprotein A-I determined by quantitative immunoelectrophoresis in delipidated fractions. It was found that the distribution of apolipoprotein A-I in lymph was shifted towards larger particles when compared to its distribution in plasma.

ORIGIN OF BILIARY CHOLESTEROL AND LECITHIN IN THE RAT: CONTRIBUTION OF NEW SYNTHESIS AND PREFORMED HEPATIC STORES. S.J. Robins, H. Brunengraber (Dept. of Med., Veterans Admin. Med. Center, Boston, MA 02130, and Dept. of Nutr. and Food Sci., Massachusetts Inst. of Tech., Cambridge, MA 02139) *J. Lipid Res.* 23(4):604-608 (1982). The contribution of de novo synthesis to the secretion of cholesterol and lecithin in bile was assessed in isolated rat liver, perfused with a lipid-free medium. Cholesterol and lecithin synthesis were measured by the incorporation of tritiated water and [14 C]choline, respectively. Taurocholate stimulated the secretion of biliary lipids to the same extent in perfused livers and in live rats. During the first hour of perfusion, and when hepatic synthesis was active, newly synthesized cholesterol accounted for about 10% of biliary cholesterol and newly synthesized lecithin for 3% of biliary lecithin. Fasting reduced the contribution of newly synthesized cholesterol in bile to less than 1% but did not change the rate of biliary cholesterol secretion. After 2 hours of perfusion, newly synthesized biliary cholesterol accounted for only 4% of total hepatic sterol synthesis. Biliary lecithin, synthesized by choline incorporation accounted for only 7% of newly synthesized hepatic lecithin. We conclude that new synthesis makes only a small contribution to biliary cholesterol and lecithin secretion, and that, in the absence of perfusate lipids, both biliary cholesterol and lecithin must be predominantly mobilized from a preformed hepatic pool.

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