

# Abstracts

## Biochemistry and nutrition

CHARACTERIZATION OF THE MULTIPLE-CHAMBER PERIFUSED FAT CELL SYSTEM. D. O. Allen, K. J. Long, and J. T. Majors (Dept. of Pharmacology, Univ. of South Carolina, School of Med., Columbia, SC 29208) *J. Lipid Res.* 20(8), 1036-40 (1979). A multiple-chamber perifused fat cell system is described. Six chambers containing fat cells were perfused in parallel with buffer. Perfusate was collected for assay of glycerol as an index of lipolytic rates and cells in each chamber can be taken for analysis of biochemical intermediates. The system is so designed that drugs can be infused into the buffer and equally distributed in each chamber or can be individually infused into the buffer to one chamber, allowing for six different conditions to be tested in the same population of fat cells. The time and distribution characteristics of infused material are described. Time relationships are described for isoproterenol and glycerol release and for cyclic AMP levels in the fat cells, and the dose-response relationship between isoproterenol and glycerol release is shown.

PHOSPHOLIPASE D FROM SAVOY CABBAGE: PURIFICATION AND PRELIMINARY KINETIC CHARACTERIZATION. T.T. Allgyer and M.A. Wells (Dept. of Biochem., College of Med., Univ. of Arizona, Tucson, AZ 85724) *Biochemistry* 18(24), 5348-53 (1979). Phospholipase D has been purified 680-fold from an acetone powder of savoy cabbage in an overall yield of 30%. The purification involves solubilization of the acetone powder in a  $Ca^{2+}$ -containing buffer and subsequent ammonium sulfate fractionation. Gel filtration of Sephadex G-200 and hydrophobic affinity chromatography using a  $\alpha$ -aminopropane-agarose gel complete the purification. The two chromatographic steps were conducted in buffers containing 50% ethylene glycol, which was necessary in order to maintain stability of the enzyme. Purity was established on the basis of gel electrophoresis and ultra-centrifugation. A preliminary kinetic characterization of the enzyme was carried out by using lecithins with short-chain fatty acids below the critical micelle concentration. A complex series of results were obtained which demonstrated the following. (1) The enzyme is quite sensitive to ionic strength, being inhibited at high ionic strength. (2) The pH optimum depends on the concentration of  $Ca^{2+}$  used in the assay. At 0.5 mM  $Ca^{2+}$  the pH optimum is 7.25, but it is 6.0 at 50 mM  $Ca^{2+}$ . (3) The effect of substrate concentration at a given pH and ionic strength did not show simple hyperbolic kinetics but regions of parabolic and hyperbolic kinetics.

BIOPOTENCIES IN RATS OF SEVERAL FORMS OF ALPHATOCOPHEROL. S.R. Ames (Biochem. Res. Lab., Health and Nutr. Res. Divs., Res. Labs., Tennessee Eastman Com., Div. of Eastman Kodak Com., Rochester, NY 14603) *J. Nutr.* 109(12), 2198-204 (1979). The biopotencies of several forms of vitamin E were determined by the rat fetal-resorption bioassay. RR- $\alpha$ -tocopheryl acetate compared with 2-*ambo*- $\alpha$ -tocopheryl acetate had a mean relative potency (RP) of 1.66, significantly higher than the currently accepted value of 1.36. RR- $\alpha$ -tocopheryl hydrogen succinate compared with 2-*ambo*- $\alpha$ -tocopheryl acetate had a mean RP of 1.125, significantly lower than the currently accepted value of 1.21. RRR- $\alpha$ -tocopherol compared with 2-*ambo*- $\alpha$ -tocopherol had a mean RP of 1.31, not significantly different from the currently accepted value of 1.36. Some preparations of *all-rac*- $\alpha$ -tocopheryl acetate compared with 2-*ambo*- $\alpha$ -tocopheryl acetate had a mean RP of 0.81, and compared with RRR- $\alpha$ -tocopheryl acetate had a mean RP of 0.52. Both RP values are significantly lower than the currently accepted values of 1.00 and 0.735 (the reciprocal of 1.36) respectively. Similar biological activities were obtained for oily and dry preparations of the same forms of  $\alpha$ -tocopheryl acetate. A basic assumption of stoichiometric equivalence for the acetate and hydrogen succinate of RRR- $\alpha$ -tocopherol, inherent in the currently accepted values, was shown to be incorrect. The results show that the unit/weight relationships for the various forms of vitamin E currently assigned by the National Formulary have not been validated using a bioassay based on biological function.

THE DIELECTRIC CONSTANT OF PHOSPHOLIPID BILAYERS AND THE PERMEABILITY OF MEMBRANES TO IONS. J.P. Dilger, S.G.A. McLaughlin, T. J. McIntosh, and S.A. Simon (Dept. of Physiology and Biophysics, State Univ. of New York, Stony Brook, NY 11794) *Science* 206(4423), 1196-(1979). The Born charging equation predicts that the permeability of a phospholipid

bilayer membrane to ions should depend markedly on the dielectric constant of the membrane. Increasing the dielectric constant of an artificial bilayer increases its permeability to perchlorate or thiocyanate by a factor of 1000, to a value comparable to that of mitochondrial membrane.

POLYPHOSPHORYLATED GLYCEROLIPIDS MIMIC ADRENOCORTICOTROPIN-INDUCED STIMULATION OF MITOCHONDRIAL PREGNENOLONE SYNTHESIS. R.V. Farese and A.M. Sabir (James A. Haley Veterans Admin. Med. Center and Dept. of Med., Univ. of South Florida College of Med., Tampa, FL 33612) *Biochim. Biophys. Acta* 575(2), 299-304 (1979). As with adrenocorticotropin pretreatment *in vivo*, addition of cardiolipin *in vitro* enhances adrenal mitochondrial pregnenolone synthesis and apparent binding of cholesterol to cytochrome P-450<sub>sc</sub>. These effects are relatively specific for glycerolipids containing two or more phosphate radicals in the polar head group, and changes in such phospholipids or comparably acting substances may play a role in mediating adrenocorticotropin- or other hormone-induced effects on membrane-associate enzymes.

INHIBITION OF THE IRON-CATALYSED FORMATION OF HYDROXYL RADICALS FROM SUPEROXIDE AND OF LIPID PEROXIDATION BY DESFERRIOXAMINE. J.M.C. Gutteridge, R. Richmond and B. Halliwell (National Institute for Biol. Standards and Control, Holly Hill, Hampstead, London NW3 6RB, U. K.) *Biochem. J.* 184(2), 469-72 (1979). The peroxidation of membrane phospholipids induced *in vitro* by ascorbic acid or by dialuric acid (hydroxybarbituric acid) does not occur in the absence of traces of metal ions. Peroxidation induced by adding iron salts to phospholipids can either be promoted or inhibited by the chelators EDTA, diethylenetriaminepenta-acetic acid and bathophenanthroline-sulphonate, depending on the ratio [chelator]/[iron salt]. The iron chelator desferrioxamine inhibits peroxidation at all concentrations tested, and it also inhibits the iron-catalysed formation of hydroxyl radicals (OH $\cdot$ ) from superoxide (O $_2^{\cdot-}$ ). Since desferrioxamine is approved for clinical use, it might prove a valuable tool in the treatment of inflammation, poisoning by autoxidizable molecules and radiation damage.

ASSAY FOR VITAMIN D<sub>2</sub> AND VITAMIN D<sub>3</sub> IN PLASMA OF DAIRY COWS: CHANGES AFTER MASSIVE DOSING OF VITAMIN D<sub>3</sub>. R.L. Horst and E.T. Littledike (Nat'l Animal Dis. Center, Sci. and Ed. Admin., Ag. Res., P.O. Box 70, Ames, IA 50010) *J. Dairy Sci.* 62(11), 1746-51 (1979). A sensitive, precise assay for vitamin D in plasma is described. Three to five milliliters of plasma were extracted with methanol: methylene chloride (2:1). The lipid extract was chromatographed on Sephadex LH-20<sup>®</sup> and then on Lipidex-5000 columns. After high pressure liquid chromatography with a reverse phase chromatographic system, vitamin D<sub>2</sub> and vitamin D<sub>3</sub> were quantitated by ultraviolet absorbance. We used this assay system for monitoring daily changes of vitamin D<sub>3</sub> in plasma of two Jersey cows after four intramuscular doses (15 x 10<sup>6</sup> IU) of vitamin D<sub>3</sub> administered at weekly intervals. Phosphorus in plasma increased sharply to a plateau at 9.5 mg/100 ml during the week after the second vitamin D<sub>3</sub> injection and returned to normal (4.5 mg/100 ml) at the end of the experiment. Calcium, however, gradually increased to 14.0 mg/100 ml 20 days after the fourth vitamin D<sub>3</sub> injection. Both animals remained hypercalcemic (calcium 11.5 mg/100 ml) during the experiment.

INHIBITION OF HEPATIC LIPOGENESIS BY 2-TETRADECYLGLYCIDIC ACID. S.A. McCune, T. Nomura, and R.A. Harris (Department of Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46223) *Lipids* 14(10), 880-2 (1979). 2-Tetradecylglycidic acid (TDGA), a hypoglycemic agent, has been found to be a very effective inhibitor of *de novo* fatty acid synthesis by isolated hepatocytes. A comparison was made between the effectiveness of TDGA and 5-(tetradecyloxy)-2-furoic acid (TOFA), a hypolipidemic agent, on the metabolic processes of isolated hepatocytes. These compounds are structurally related and both inhibit fatty acid synthesis; however, they have opposite effects from each other on the oxidation and esterification of fatty acids. TDGA inhibits whereas TOFA stimulates fatty acid oxidation. TDGA stimulates whereas TOFA inhibits fatty acid esterification.

PROSTAGLANDIN, THROMBOXANE, AND 12-HYDROXY-5, 8, 10, 14-EICOSATETRAENOIC ACID PRODUCTION BY IONOPHORE-STIMULATED RAT SEROSAL MAST CELLS. L.J. Roberts, II, RA. Lewis, J.A. Oates and K.F. Austen (Dept. of Pharmacology, Vanderbilt Univ. Med. Center, Nashville, TN 37232) *Biochim. biophys. Acta* 575(2),185-92 (1979). Production of several metabolites of arachidonic acid by purified rat serosal mast cells in response to stimulation with the ionophore A23187 was assessed by stable isotope dilution assay using gas chromatography-mass spectrometry. Compounds quantified were prostaglandins D<sub>2</sub>, E<sub>2</sub>, F<sub>2α</sub>, 6-keto-F<sub>1α</sub>, thromboxane B<sub>2</sub>, and 12-hydroxy-5, 8, 10, 14-eicosatetraenoic acid. Mast cells incubated at 37 C for 30 min without ionophore produced measurable quantities of all metabolites assayed. 4 μM A<sub>23187</sub> resulted in substantial increased synthesis of all metabolites compared to control cells. Of the metabolites quantified, prostaglandin D<sub>2</sub> and prostacyclin were the major products derived from arachidonic acid in ionophore-stimulated rat mast cells.

ACETATE IS THE PREFERRED SUBSTRATE FOR LONG-CHAIN FATTY ACID SYNTHESIS IN ISOLATED SPINACH CHLOROPLASTS. P.G. Roghan, R. Holland and C.R. Slack (Plant Physiology Div., D.S.I.R., Private Bag, Palmerston North, New Zealand) *Biochem. J.* 184(3),565-9 (1979). Commercially available [2-<sup>14</sup>C] pyruvate and [2-<sup>14</sup>C] malonate were found to contain 3-6% (w/w) of [<sup>14</sup>C] acetate. The contaminating [<sup>14</sup>C] acetate was efficiently utilized for fatty acid synthesis by isolated chloroplasts, whereas the parent materials were poorer substrates. Maximum incorporation rates of the different substrates examined were (ng<sup>14</sup>-atoms of c/h per mg of chlorophyll): [1-<sup>14</sup>C] acetate, 2676; [2-<sup>14</sup>C] pyruvate, 810; H<sup>14</sup>CO<sub>3</sub><sup>-</sup>, 355; [2-<sup>14</sup>C] malonate, 19. Products of CO<sub>2</sub> fixation were probably not a significant carbon source for fatty acid synthesis in the presence of exogenous acetate.

LINOLEATE AND α-LINOLENATE SYNTHESIS BY ISOLATED SPINACH (*SPINACIA OLERACEA*) CHLOROPLASTS. P.G. Roughtan, J.B. Mudd and T.T. McManus (Dept. of Biochem., Univ. of California Riverside, CA 92521) *Biochem. J.* 184(3),571-4 (1979). Diacylgalactosylglycerol synthesis was a prerequisite for the incorporation of [1-<sup>14</sup>C]-acetate into linoleate and α-linolenate of isolated spinach (*Spinacia oleracea*) chloroplasts. Oleate at position 1 of diacylgalactosylglycerol was desaturated to linoleate and α-linolenate both in the light and in the dark. Some desaturation of palmitate was also observed after prolonged incubations.

PARTICIPATION OF THE MEMBRANE IN THE SIDE CHAIN CLEAVAGE OF CHOLESTEROL. RECONSTITUTION OF CYTOCHROME P-450<sub>SCC</sub> INTO PHOSPHOLIPID VESICLES. D.W. Seybert, J.R. Lancaster, Jr., J.D. Lambeth and H. Kamin (Dept. of Biochem., Duke Univ. Med. Center, Durham, NC 27710) *J. Biol. Chem.* 254(23),12088-98 (1979). Cytochrome P-450<sub>SCC</sub> can be reconstituted into a phospholipid bilayer in the absence of added detergent by incubation of purified hemoprotein with preformed phosphatidylcholine vesicles. Salt effects demonstrate that the primary interaction between the cytochrome and phospholipid vesicles is hydrophobic rather than ionic; in contrast, neither adrenodoxin reductase nor adrenodoxin will bind to phosphatidylcholine vesicles by hydrophobic interactions. The results indicate that the cholesterol binding site on vesicle-reconstituted cytochrome P-450<sub>SCC</sub> is in communication with the hydrophobic bilayer of the membrane. The reducibility of vesicle-reconstituted cytochrome P-450<sub>SCC</sub> as well as spectrophotometric and activity titration experiments show that all of the reconstituted cytochrome P-450<sub>SCC</sub> molecules possess an adrenodoxin binding site which is accessible from the exterior of the vesicle. Activity titrations with adrenodoxin reductase also demonstrate that a ternary or quaternary complex among adrenodoxin reductase, adrenodoxin, and cytochrome P-450<sub>SCC</sub> is not required for catalysis, a finding consistent with our proposed mechanism of steroidogenic electron transport in which adrenodoxin acts as a mobile electron shuttle between adrenodoxin reductase and cytochrome P-450.

COMPOSITION OF POLAR LIPIDS IN CARROT ROOTS. J. Soimajarvi and R.R. Linko (Dept. of Biology, Univ. of Jyväskylä SF 40100 Jyväskylä, Finland) *J. Agric. Food Chem.* 27(6),1279-81 (1979). Lipids extracted from carrot roots have been fractionated on silicic acid column into neutral lipids (NL), glycolipids (GL), and phospholipids (PL), and the composition of the polar lipids have been studied by chemical analysis after thin-layer chromatographic (TLC) development. In root samples of different origin the total lipid content at harvest varied from 225 to 340 mg/100 g of fresh root, but the percent composition of the three main lipid fractions was nearly similar: NL represented over 60% of the total lipid and the percentages of GL and PL were respectively 13-21% and 16-21%. The most abundant lipid classes in the PL fraction were phosphatidylcholines and phosphatidylethanolamines which

together amounted to 65% of this fraction. The PL fraction contained 1.0% aldehydogenic lipids. The main part of GL consisted of digalactosyl and monogalactosyl diglycerides. The quantitative composition of polar lipids was in general very similar to that in many other nonphotosynthetic plant storage tissues.

ENTRAPMENT OF PROTEINS IN PHOSPHATIDYLCHOLINE VESICLES. G. Adrian and L. Huang (Dept. of Biochem., Univ. of Tennessee, Knoxville, TN 37916) *Biochemistry* 18(25),5610-4 (1979). The trapping efficiency of globular proteins in four different types of phosphatidylcholine vesicles was systematically studied. Vesicles were generated in a mixture of <sup>125</sup>I-labeled proteins of various molecular weights. The trapped proteins were separated from untrapped proteins by gel filtration and ultrafiltration and subsequently analyzed by gel electrophoresis and autoradiography. Entrapment of proteins was demonstrated by their resistance to trypsin digestion. The relative amount of each entrapped protein species was then compared to that of the original protein solution. In multilamellar vesicles and large unilamellar vesicles, proteins of molecular weight up to 97 000 had the same trapping efficiency as sucrose. In small unilamellar vesicles generated by either sonication or ethanol injection, however, the relative trapping efficiency of protein decreased progressively as the molecular weight of the protein became greater. For example, the trapping efficiency of α-amylase (M<sub>r</sub> 97 000) was only half of that for sucrose. The apparent decrease in trapping efficiency with the protein's molecular weight in small unilamellar vesicles can be accounted for by the combination of the bound water layer at the vesicle's internal surface and the steric hindrance when protein is captured during vesicle formation.

CHANGES IN THE PHOSPHOLIPID COMPOSITION OF MICRO-SOMAL MEMBRANES OF DYSTROPHIC HAMSTERS. H.A. Barakat, D.R. Johnson and D.S. Kerr (Biochem. Dept., East Carolina Univ. Med. School, Greenville, NC 27834) *Proc. Soc. Exp. Biol. & Med.* 163(1),167-70 (1980). Analysis of liver microsomal phospholipids of dystrophic and normal hamsters showed changes in the content and fatty acid composition of these compounds in the diseased animals. The total phospholipid, phosphatidylcholine, and phosphatidylethanolamine concentrations were elevated. Determination of the fatty acid composition revealed a decrease in the concentration of monounsaturated fatty acids with a concomitant increase in the concentration of polyunsaturated fatty acids. It is concluded that these changes may affect the physical nature of membranes. Such a change in the physical state of the membrane may affect the orientation of membrane proteins, which in turn may cause alterations in enzymatic activity.

GROWTH-RELATED LIPID PEROXIDATION IN TUMOUR MICRO-SOMAL MEMBRANES AND MITOCHONDRIA. G.M. Bartoli and T. Galeotti (Inst. of General Pathology, Catholic Univ., S. Cuore, Via Pineta Sacchetti 644, 00168 Roma Italy) *Biochim. Biophys. Acta* 574(3),537-41 (1979). Microsomes and mitochondria isolated from Morris hepatomas 3924A (fast-growing) and 44 (slow-growing) and Ehrlich ascites tumour cells exhibit a NADPH-dependent peroxidation of endogenous lipids lower than that of the corresponding fractions from rat liver. Moreover, the O<sub>2</sub><sup>-</sup> and ascorbate-dependent lipid peroxidations are decreased in microsomes from the two Morris hepatomas. The peroxidative activity appears to be inversely related to the growth rate of the tumours. It is suggested that the low susceptibility of tumour membranes to peroxidative agents may be a factor responsible for the high mitotic activity of this tissue.

NUTRITIONAL FACTORS AFFECTING QUANTITY AND QUALITY OF CARCASS FAT IN CHICKENS. I. Bartov (Dept. of Poultry Sci., Univ. of Georgia, Athens, Georgia 30602) *Fed. Proc.* 38(12),2627-30 (1979). Nonpathological fattening of a bird occurs when the amount of energy consumed exceeds its requirements for maintenance and growth. Dietary energy and protein levels, particularly the ratio of these two, are the main dietary factors affecting fatness. Consumption of diets low in protein results in excess energy intake and an increased hepatic lipogenesis. Excess protein has the opposite effect. It also increases the energy expenditure required to dispose of excess amino acids in the body. Severe deficiency of a specific amino acid does not increase fattening. The degree of fattening, particularly of the liver, induced by corticosterone injection is greater in birds fed diets containing a wide energy-to-protein ratio in comparison to a narrow ratio. The content of dietary fat per se does not affect carcass fat concentrations although it alters the rate of liver fatty acid synthesis. The dietary fatty acid composition affects the composition of tissue fatty acids. Consumption of diets containing vegetable oils or high in protein increases the degree of unsaturation of tissue fat and thereby its susceptibility to oxidation. Dietary dl-α-tocopheryl acetate increases the stability of the lipids of adipose and muscle

tissue of chicks with relatively saturated body fat, but the dietary effectiveness of this vitamin in improving the stability of tissues of birds having relatively unsaturated fat is limited.

**SOURCE OF THE CHOLESTEROL ESTER ACCUMULATED IN MONKEY ARTERIAL SMOOTH MUSCLE CELLS GROWN IN HYPERLIPEMIC SERUM.** S.R. Bates (Dept. of Pathology and Specialized Center of Res. in Atherosclerosis, Univ. of Chicago, Chicago, IL) *Circ. Res.* 45(6),821-8 (1979). I studied various sources of cellular cholesterol ester to determine the origin of the esters that accumulated in monkey smooth muscle cells exposed to hyperlipemic serum. The movement of free cholesterol between the serum and the cells and its esterification by the smooth muscle cells were followed by means of a double-label procedure. Both uptake and efflux of free cholesterol were nearly linear over a 40-hour period. Approximately one-third of the total cellular cholesterol ester was derived from the esterification of free cholesterol taken up from the hyperlipemic serum in the medium, and one-third originated from the esterification of the free cholesterol present in the cells at zero time. Cholesterol esters of the hyperlipemic serum accounted for the major portion of the final third. The results suggest that cellular esterification of free cholesterol may be of major importance to the increase in the cholesterol ester content of arterial cells exposed to hyperlipemic serum, since this process provided at least 60% of the total cholesterol esters accumulated.

**A MATHEMATICAL RELATIONSHIP BETWEEN THE FATTY ACID COMPOSITION OF THE DIET AND THAT OF THE ADIPOSE TISSUE IN MAN.** A.C. Beynen, R.J.J. Hermus, and J.G.A.J. Hautvast (Laboratory of Veterinary Biochem., State Univ. of Utrecht, Biltstraat 172, Utrecht) *Am. J. Clin. Nutr.* 33(1),81-5 (1980). Based on literature data, the hypothesis is advanced that in human subjects a direct mathematical relationship exists between the average fatty acid composition of the habitual diet and that of the lipid stores of subcutaneous adipose tissue. Since the half-life of adipose tissue fatty acids in man is in the order of 600 days, the fatty acid pattern of depot fat provides a qualitative measure of the fat intake over a period of 2 to 3 years. It is concluded that in long-term experimental and epidemiological nutritional surveys the adipose tissue fatty acid pattern of the subjects is a useful index of the average composition of their habitual dietary fat.

**FATTY ACID COMPOSITION OF HEART CELLS EXPOSED TO THERMALLY OXIDIZED FATS.** R.P. Bird and J.C. Alexander (Department of Nutrition, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1) *Lipids* 14(10), 836-41 (1979). Corn oil and olive oil were thermally oxidized, and the free fatty acids from the fresh fats, and from the distillable non-urea-adductable (DNUA) fractions of the thermally oxidized fats were prepared. These were added as emulsions to the medium of primary cultures of heart endothelial and muscle cells from neonatal rats. After exposure for 24 hr, the fatty acid composition of the triacylglycerol (TC) and phospholipid (PL) fractions of the cells was determined. Reflecting the nature of the fat used, the corn oil treatment produced relatively higher concentrations of linoleic acid in the TG and PL fractions compared to the olive oil treatment, in which case the oleic acid level was influenced. Treatment of the cultured cells with components derived from oxidized corn oil or oxidized olive oil resulted in lower concentrations of linoleic and arachidonic acids in the PL moieties compared to the fresh fat controls. However, there were marked increases in arachidonic acid in the TG fractions of both the endothelial and muscle cells. These changes due to the DNUA from thermally oxidized fats indicate a distinct metabolic response to the derivatives formed during thermal oxidation of the fats.

**DEPENDENCE OF THE EFFECTS OF DIETARY CHOLESTEROL AND EXPERIMENTAL CONDITIONS ON SERUM LIPIDS IN MAN. III. THE EFFECT ON SERUM CHOLESTEROL OF REMOVAL OF EGGS FROM THE DIET OF FREE-LIVING HABITUALLY EGG-EATING PEOPLE.** D.C. Bronsgeest-Schoute, R.J.J. Hermus, G.M. Dallinga-Thie, and J.G.A.J. Hautvast (Dept. of Human Nutr., Agric. Univ., 6703 BC Wageningen, The Netherlands) *Amer. J. Clin. Nutr.* 32(11),2193-7 (1979). Forty-four healthy free living volunteers were used to study the effect of the removal of eggs from a habitual egg-rich diet. The subjects, recruited by advertising, normally consumed at least 1 egg per day. During the 3-week experimental period they were not allowed to eat any eggs or products containing large amounts of eggs, except cakes and tarts. Elimination of eggs from a habitual egg-rich diet did result in a small but significant decrease in serum cholesterol levels in all subjects. The results indicate that a very variable response is present in a human population toward dietary cholesterol. More research seems to be necessary to describe and select the population of hyperresponders, the subjects who are more sensitive to changes in dietary cholesterol, and the hyporesponders. The re-

sults moreover indicate that effects of dietary changes in a free-living population are much smaller than can be accomplished in populations under controlled conditions.

**DEPENDENCE OF THE EFFECTS OF DIETARY CHOLESTEROL AND EXPERIMENTAL CONDITIONS ON SERUM LIPIDS IN MAN. II. EFFECTS OF DIETARY CHOLESTEROL IN A LINOLEIC ACID-POOR DIET.** D.C. Bronsgeest-Schoute, R.J.J. Hermus, G.M. Dallinga-Thie, and J.G.A.J. Hautvast (Dept. of Human Nutr., Agric. Univ., 6703 BC Wageningen, The Netherlands) *Amer. J. Clin. Nutr.* 32(11),2188-92 (1979). In this experiment the effect of dietary cholesterol on serum cholesterol levels was tested in a linoleic acid-poor diet. In a cross-over design 34 healthy students received a linoleic acid-poor diet for 6 weeks at two levels of dietary cholesterol for 3 weeks each. It is concluded that the effect of dietary cholesterol is clearly independent on the type of fat present in the diet. Eggs will have a rather important elevating effect in a saturated fat diet, which is the more deleterious as the saturated fat already induces high cholesterol levels. However, linoleic acid-rich fat reduces serum cholesterol levels markedly, while the opposing effect of dietary cholesterol in this case is much smaller. The results of this study emphasize the importance of considering the complexity and interrelations of all constituents of a diet in affecting health parameters.

**DEPENDENCE OF THE EFFECTS OF DIETARY CHOLESTEROL AND EXPERIMENTAL CONDITIONS ON SERUM LIPIDS IN MAN. I. EFFECTS OF DIETARY CHOLESTEROL IN A LINOLEIC ACID-RICH DIET.** D.C. Bronsgeest-Schoute, J.G.A.J. Hautvast, and R.J.J. Hermus (Dept. of Human Nutrition, Agric. Univ., 6703 BC, Wageningen, The Netherlands) *Amer. J. Clin. Nutr.* 32(11),2183-7 (1979). In this experiment the effect of dietary cholesterol in a linoleic acid-rich diet on serum cholesterol was tested. In a cross-over design 41 young healthy students received a linoleic acid-rich diet for 4 weeks at two levels of dietary cholesterol. The diet contained 14 to 15 energy % linoleic acid. The high cholesterol diet was obtained by adding two egg yolks a day to the rations. Supplementation of the linoleic acid-rich diet with the egg yolk cholesterol caused a significant rise of serum cholesterol of about 11 mg/100 ml (0.29 mmole/liter). The dietary cholesterol did not influence serum triglyceride levels. The influence on serum cholesterol was much less than expected, based on several predictive formulas. It is concluded that the presence of a high content of linoleic acid in the diet reduces the effect of dietary cholesterol on serum cholesterol if the cholesterol is provided as egg yolk.

**SERUM CHOLESTEROL AND HIGH DENSITY LIPOPROTEIN-CHOLESTEROL IN CORONARY PATIENTS AND HEALTHY PERSONS.** D. Brunner, J. Weisbort, K. Loebl, S. Schwartz, S. Altman, J.E. Bearman and S. Levin (Med. Dept. A, Govt. Hospital, Jaffa, Israel) *Atherosclerosis* 33(1),9-16 (1979). Serum cholesterol (CH) and high density lipoprotein-cholesterol (HDL-CH) were determined in 154 male and in 68 female post-myocardial infarction (MI- patients and in 2706 healthy males and 1888 healthy females. CH values showed no significant differences between healthy subjects and MI patients (except in males 35-44 years old). HDL-CH values were significantly lower in MI patients than in healthy subjects. In addition, in subgroups with equal CH values, MI patients had significantly lower HDL-CH than healthy people. Healthy females had higher HDL-CH than healthy males with same CH levels, but there was no difference in HDL-CH between male and female coronary patients with equal CH values. The term HDL-CH%, indicating the percentage of total CH in the HDL, seems preferable to HDL-CH mg/100 ml, because it is independent of the level of CH. In the population surveyed, HDL-CH showed itself as a more reliable indicator for ischemic heart disease than CH. The hypothesis is advanced that the first step in the development of an atherogenic lipoprotein pattern is a re-distribution of CH between HDL on the one hand, and LDL plus VLDL on the other hand, the second step only being an increase of CH, mainly in the low density lipoprotein fractions.

**IN VITRO LIPID METABOLISM IN THE RAT PANCREAS. I. BASAL LIPID METABOLISM.** P. Calderon, J. Furnelle and J. Christophe (Dept. of Biochem. and Nutr., Med. Schl., Universite Libre de Bruxelles, Waterloo Boulevard 115, B-1000 Brussels Belgium) *Biochim. Biophys. Acta* 574(3),379-90 (1979). The in vitro basal lipid metabolism of rat pancreatic fragments was compared with that in adipose tissue fragments and liver slices [ $^{14}\text{C}$ ]. Acetate added to the media was mostly incorporated into palmitic acid and to a lesser extent into oleic acid. In addition, pancreatic tissue exhibited a marked capacity for elongation of polyunsaturated fatty acids by [ $^{14}\text{C}$ ] acetate and resulting desaturation when compared to adipose tissue and liver. Data obtained in the presence of [ $^{14}\text{C}$ ] glucose, [ $^{14}\text{C}$ ] palmitate and  $^3\text{H}_2\text{O}$  indicate that acetyl-CoA derived from glucose and from  $\beta$ -oxidation of fatty acids

contributed to de novo lipogenesis. Oxidation of [1-<sup>14</sup>C] palmitic acid was 9-13 times higher in the pancreas than in adipose tissue or liver when expressed on a wet weight basis. The fatty acid moiety of pancreatic glycerolipids could be derived from de novo synthesis, fatty acids added to the medium, or from fatty acids formed from the hydrolysis of endogenous lipids. The glycerol moiety could be derived either from glucose, or directly from glycerol through participation of glycerol kinase.

**IN VITRO LIPID METABOLISM IN THE RAT PANCREAS. II. EFFECTS OF SECRETAGOGUES ON FATTY ACID METABOLISM, NET LIPOLYSIS AND ATP LEVELS.** P. Calderon, J. Furnelle and J. Christophe (Dept. of Biochem. and Nutr., Med. Schl., Université Libre de Bruxelles, Waterloo Boulevard, B-1000 Brussels Belgium) *Biochim. Biophys. Acta* 574(3), 391-403 (1979). The concentration of carbamylcholine, bombesin, pancreozymin, pentagastrin and secretin evoking a similar 4-5-fold maximal increase in amylase secretion from rat pancreatic fragments were  $3 \cdot 10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$ ,  $3 \cdot 10^{-6}$ , and  $3 \cdot 10^{-6}$  M, respectively. When used at their optimal concentrations, carbamylcholine, bombesin, pancreozymin, and pentagastrin lowered pancreatic ATP levels by 18-26% and increased net release of free fatty acids by 68-105%. The spectrum of fatty acids labeled with [1-<sup>14</sup>C] acetate indicated an inhibition of the malonic acid pathway whereas the elongation of polyenoic fatty acids was unaltered.

**IN VITRO LIPID METABOLISM IN THE RAT PANCREAS. III. EFFECTS OF CARBAMYLCHOLINE AND PANCREOZYMIN ON THE TURNOVER OF PHOSPHATIDYLINOSITOLS, 1,2-DIACYLGLYCEROLS AND PHOSPHATIDYLCHOLINES.** P. Calderon, J. Furnelle and J. Christophe (Dept. of Biochem. and Nutr., Med. Schl., Université Libre de Bruxelles, Waterloo Boulevard 115, B-1000 Brussels, Belgium) *Biochim. Biophys. Acta* 574(3), 404-13 (1979). The turnover of phosphatidylinositols and other glycerolipids was examined in rat pancreatic fragments incubated in the presence of carbamylcholine and pancreozymin used at a concentration inducing maximal  $\alpha$ -amylase hypersecretion. Variations in the percent distribution of <sup>14</sup>C among fatty acids and in specific activity of individual fatty acids in each lipid class suggested that the secretagogues reduced selection of newly synthesized 1,2-diacylglycerols which occurred in the resting state before their incorporation into phosphatidylinositols. Increased rate of incorporation of [1-<sup>14</sup>C] palmitate, [1-<sup>14</sup>C] linoleate, [1-<sup>14</sup>C] arachidonate and [1(3)(n)-<sup>3</sup>H] glycerol into phosphatidylinositols was detrimental to phosphatidylcholines. In the present paper, the last of a series of three, the two secretagogues carbamylcholine and pancreozymin were tested at concentrations of  $3 \cdot 10^{-6}$  M and  $10^{-8}$  M, respectively, inducing maximal  $\alpha$ -amylase hypersecretion from rat pancreatic fragments. The turnover of each part of phosphatidylinositols was investigated using <sup>32</sup>P<sub>i</sub>, [1(3)(n)-<sup>3</sup>H] glycerol, *myo*-[2-<sup>3</sup>H] inositol, as well as exogenous and endogenous fatty acids. This turnover was compared with that of other glycerolipids and its calcium dependence was examined.

**STEROL SYNTHESIS: STUDIES OF THE METABOLISM OF 14 $\alpha$ -METHYL-5 $\alpha$ -CHOLEST-7-EN-3 $\beta$ -OL.** J.T. Chan, T.E. Spike, S.T. Trowbridge and G.J. Schroepfer, Jr. (Depts. of Biochemistry and Chemistry, Rice Univ., Houston, TX 77001) *J. Lipid Res.* 20(8), 1007-19 (1979). [3 $\alpha$ -<sup>3</sup>H] 14 $\alpha$ -Methyl-5 $\alpha$ -cholest-7-en-3 $\beta$ -ol has been prepared by chemical synthesis. The metabolism of this compound has been studied in the 10,000 g supernatant fraction of liver homogenates of female rats. Efficient conversion to cholesterol was observed. Other labeled compounds recovered after incubation of [3 $\alpha$ -<sup>3</sup>H] 14 $\alpha$ -methyl-5 $\alpha$ -cholest-7-en-3 $\beta$ -ol with the enzyme preparations include the unreacted substrate, 5 $\alpha$ -cholesta-7,14-dien-3 $\beta$ -ol, 5 $\alpha$ -cholesta-8,14-dien-3 $\beta$ -ol, cholesta-5,7-dien-3 $\beta$ -ol, 5 $\alpha$ -cholest-8(14)-en-3 $\beta$ -ol, 5 $\alpha$ -cholest-8-en-3 $\beta$ -ol, and 5 $\alpha$ -cholest-7-en-3 $\beta$ -ol. In addition, significant amounts of incubated radioactivity were recovered in sterol esters. The steroidal components of these esters were found to contain labeled 14 $\alpha$ -methyl-5 $\alpha$ -cholest-7-en-3 $\beta$ -ol, 5 $\alpha$ -cholesta-8,14-dien-3 $\beta$ -ol, 5 $\alpha$ -cholesta-7,14-dien-3 $\beta$ -ol, 5 $\alpha$ -cholest-8-en-3 $\beta$ -ol, 5 $\alpha$ -cholest-7-en-3 $\beta$ -ol, and cholesterol.

**UTILIZATION OF ENDOGENOUS DIACYLGLYCEROL FOR THE SYNTHESIS OF TRIACYLGLYCEROL, PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE BY LIPID PARTICLES FROM BAKER'S YEAST (*SACCHAROMYCES CEREVISIAE*).** K. Christiansen (Dept. of Biochem. C, Panum Inst., Univ. of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N Denmark) *Biochim. Biophys. Acta* 574(3), 448-60 (1979). The activity of the enzymes diacylglycerol acyltransferase cholinephosphotransferase and ethanolaminephosphotransferase have been measured in a lipid particle preparation from baker's yeast with endogenous 1,2-diacylglycerol as substrate. For all three enzymes the rate of diacylglycerol utilization was established with respect to substrate and Mg<sup>2+</sup> concentration. The conversion of diacylglycerol into triacylglycerol in the presence of CDPcholine and

CDPethanolamine, and the synthesis of phospholipids in the presence of acyl-CoA either added or generated in situ were studied. When the necessary substrates for formation of acyl-CoAs in situ were added a decrease in both cholinephosphotransferase and ethanolaminephosphotransferase activity was observed. This inhibition was shown to be due to ATP and might be explained as a result of chelation of the Mg<sup>2+</sup>, a necessary activator of both the choline- and the ethanolaminephosphotransferase.

**ACUTE CONTROL OF FATTY ACID SYNTHESIS BY CYCLIC AMF IN THE CHICK LIVER CELL: POSSIBLE SITE OF INHIBITION OF CITRATE FORMATION.** S.D. Clarke, P.A. Watkins and M.D. Lane (Dept. of Physiological Chem., Johns Hopkins Univ. School of Medicine and Nutr. Program, School of Hygiene and Public Health, Baltimore, MD 21205) *J. Lipid Res.* 20(8), 974-85 (1979). Glucagon and N, <sup>6</sup>O<sup>2</sup>-dibutyryl cyclic adenosine 3',5'-cyclic monophosphate (Bt<sub>2</sub>cAMP) inhibit fatty acid synthesis from acetate by more than 90% and prevent citrate formation in chick hepatocytes metabolizing glucose. In the presence of an inhibitor of mitochondrial pyruvate transport lactate accumulation is enhanced, but continues to be lowered 50% by Bt<sub>2</sub>cAMP. The activity of phosphofructokinase is greatly decreased in Bt<sub>2</sub>cAMP-treated cells while the activities of pyruvate kinase and acetyl-CoA carboxylase are unaffected. It appears that decreased glycolytic flux and decreased citrate formation result from depressed phosphofructokinase activity. These results implicate a second site of inhibition of fatty acid synthesis by Bt<sub>2</sub>cAMP that involves the utilization, but not the production, of cytoplasmic acetyl-CoA.

**INFLUENCE OF SELENIUM, VITAMIN E, AND ETHOXYQUIN ON LIPID PEROXIDATION IN MUSCLE TISSUES FROM FOWL DURING LOW TEMPERATURE STORAGE.** G.F. Combs, Jr. and J.M. Regenstein (Dept. of Poultry Sci. and Div. of Nutritional Sciences, Cornell Univ., Ithaca, NY 14853) *Poult. Sci.* 59(2), 347-51 (1980). The influences of various factors which affect the selenium-vitamin E status of laying hens on lipid peroxidation in muscle tissues during low temperature storage were studied. Laying hens from 32 to 56 weeks of age were fed low selenium and low vitamin E practical diets supplemented with different levels of Na<sub>2</sub>SeO<sub>3</sub>, dl- $\alpha$ -tocopherol acetate, ethoxyquin, and/or peroxidized corn oil. Vitamin E status as indicated by plasma vitamin E activity was improved by supplements of vitamin E, selenium, or ethoxyquin. Selenium status as indicated by plasma selenium-dependent glutathione peroxidase activity was improved by selenium supplementation. Incorporation of peroxidized corn oil into diets did not depress plasma vitamin E but increased plasma glutathione peroxidase when those diets contained supplemental selenium. Lipid peroxidation as indicated by the 2-thiobarbituric acid (TBA) method in muscle samples held at -20 C for up to 270 days was reduced by dietary selenium in *M. pectoralis* when corn oil was fed. Supplemental vitamin E or ethoxyquin reduced TBA values developed in *M. gastrocnemius*. Results indicate that dietary selenium and other factors affecting selenium status may be useful in retarding the development of oxidative rancidity in frozen poultry products.

**CELL TRIACYLGLYCEROL ACCUMULATION FROM VERY LOW DENSITY LIPOPROTEINS ISOLATED FROM NORMAL AND HYPERTRIGLYCERIDEMIC HUMAN SERA.** M. de la Llera, G. Rothblat and B.V. Howard (Dept. of Physiology and Biochem., The Med. College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129) *Biochim. Biophys. Acta* 574(3), 414-22 (1979). Human fibroblast cells in culture increased their intracellular triacylglycerol levels when exposed to very low density lipoproteins (VLDL) isolated from human plasma. This response was dependent on the amount of VLDL added. VLDL from normal, type IV or type V sera gave similar results. Lipoprotein lipase enhanced this intracellular triacylglycerol accumulation. It was concluded that human fibroblast cells in culture have at least two mechanisms for triacylglycerol uptake from VLDL: (1) uptake from intact lipoprotein either by surface transfer of lipoprotein lipid or internalization of the entire lipoprotein particle, and (2) re-esterification of lower glyceride and fatty acids released by lipoprotein lipase degradation of VLDL.

**REGULATION OF FATTY ACID SYNTHESIS.** W.E. Donaldson (Dept. of Poultry Sci., North Carolina State Univ., Raleigh, NC 27650) *Fed. Proc.* 38(12), 2617-21 (1979). Acetyl-CoA carboxylase and fatty acid synthetase are the two major enzymes involved in the synthesis of fatty acids in animals. The activities of both enzymes are affected by nutritional manipulations. Although acetyl-CoA carboxylase is considered generally to be the rate-limiting step in lipogenesis, there is evidence that suggests that fatty acid synthetase may become rate limiting under certain conditions. The principal support for the view that acetyl-CoA carboxylase is the rate-limiting enzyme for lipogenesis is that the activity of the enzyme is controlled by allosteric effectors that change the catalytic efficiency of

the enzyme. Until recently, the only known control of fatty acid synthetase was through changes in rate of enzyme synthesis. Data are reviewed that show that fatty acid synthetase can exist in forms possessing different catalytic activities. Thus fatty acid synthetase appears to be subject to the type of control necessary for an enzyme to serve as a regulator of the rate of a biological process over a short term.

**EFFECTS OF DIETARY CALCIUM ON BLOOD AND TISSUE LIPIDS, TISSUE PHOSPHOLIPIDS, CLACIUM AND MAGNESIUM LEVELS IN RABBITS FED DIETS CONTAINING BEEF TALLOW.** R.M. Dougherty and J.M. Iacono (Lipid Nutr. Lab., Nutr. Inst., Human Nutr. Center, Sci. and Edu. Admin., Beltsville Agr. Res. Center, Beltsville, MD 20705) *J. Nutr.* 109(11), 1934-45 (1979). Levels of lipids, calcium and magnesium in blood and tissue were examined in rabbits to determine the effects of 20% beef tallow diets containing three levels of calcium, < 0.02, 0.8 or 1.6%. In plasma, the calcium-deficient (< 0.02%) diet contributed to elevated cholesterol and phospholipid, but had no effect on triglyceride levels. Plasma calcium decreased in the calcium-deficient group and plasma magnesium decreased in the high-calcium (1.6%) group of rabbits. Lipid levels of some tissues varied with the level of dietary calcium. Cholesterol, total phospholipid, sphingomyelin and phosphatidylethanolamine were generally elevated in livers of calcium-deficient rabbits, but the individual phospholipids were decreased in skeletal muscle. Lungs of the calcium-deficient group also had lower phospholipid levels than the high-calcium group. Liver, kidneys, brain and adipose tissue triglyceride levels were highest in the high-calcium group. The calcium level of skeletal muscle was lower in the calcium-deficient group than in the high-calcium group. Calcium in brain and adipose tissue were highest in the calcium-deficient group. Except for adipose tissue, magnesium levels of the tissues studied were not affected by dietary calcium.

**CORRELATIONS BETWEEN INTRAVENOUS FAT TOLERANCE AND SERUM LIPOPROTEINS IN NORMAL AND ATHEROSCLEROTIC SUBJECTS.** M. Ericsson and S. Rössner (Dept. of Med. and King Gustaf V Res. Inst., Karolinska Hospital, Stockholm, Sweden) *Atherosclerosis* 33(1), 89-97 (1979). Triglyceride (TG) and cholesterol concentrations in the major lipoprotein fractions and the intravenous fat tolerance test (IVFTT)  $k_2$  value were determined in healthy controls (n=35), in survivors of myocardial infarction (MI, n=43) and in patients with peripheral vascular disease (PVD, n=33). MI patients had the highest lipid concentrations in the lipoproteins except for HDL-cholesterol which was 26% lower in both MI and PVD patients compared to controls. For the other lipid concentrations, PVD patients had values between MI patients and control values. A strong positive correlation was found between  $k_2$  and HDL-cholesterol. When partial regression analysis was carried out, this relationship prevailed also when the effects of both VLDL-TG and total TG were eliminated ( $r=0.32$ ,  $P < 0.001$ ). These results support the concept that the IVFTT  $k_2$  value independently gives an estimate of TG removal processes from plasma and reflects HDL activity on TG catabolism.

**EFFECTS OF DIETS OF HOMOGENEOUS SATURATED TRIGLYCERIDES ON CHOLESTEROL BALANCE IN RATS.** E.B. Feldman, B.S. Russell, F.H. Schnare, I. Morett-Rojas, B.C. Miles and E.A. Doyle (Dept. of Med., Schl. of Med., The Med. College of Georgia, Augusta, GA 30912) *J. Nutr.* 109(12), 2237-46 (1979). The effects of a diet of 10% homogeneous triglycerides of 12 to 18-carbon chain saturated fatty acids on cholesterol absorption and turnover were studied in rats. Cholesterol absorption was successively significantly less in rats fed tristearin than in groups fed tripalmitin, trimyristin and trilaurin. Lesser fatty acid absorption may explain the differences in part, since cholesterol absorption was significantly correlated with fat absorption. Cholesterol removal from plasma was fastest in rats fed tristearin. Plasma cholesterol levels were increased with the trilaurin diet although the rate of cholesterol accumulation in lymph after gavage was slower with trilaurin. Lymph triglycerides were highest with trilaurin and trimyristin diets perhaps indicating endogenous mobilization of triglyceride for lipoprotein formation. Lymph triglycerides were, however, decreased with tristearin. Sterol turnover (production, absorption plus synthesis) was increased with tristearin or trilaurin by kinetic or balance methods.

**EFFECTS OF TRISTEARIN, TRIOLEIN AND SAFFLOWER OIL DIETS ON CHOLESTEROL BALANCE IN RATS.** E.B. Feldman, B.S. Russell, F.H. Schnare, B.C. Miles, E.A. Doyle and I. Moretti-Rojas (Dept. of Med. Schl. of Med., The Med. College of Georgia, Augusta, GA 30912) *J. Nutr.* 109(12), 2226-36 (1979). Diets containing relatively homogeneous triglycerides composed of 18-carbon chain saturated, monounsaturated or polyunsaturated fatty acids were fed to rats. Cholesterol absorption and turnover were studied. Cholesterol absorption was significantly less in rats fed

tristearin than in animals fed triolein or safflower oil. Cholesterol removal from plasma was fastest in rats fed tristearin and slowest with safflower oil and triolein. Plasma cholesterol levels were lowest with tristearin and highest with safflower oil. Increased cholesterol in high density lipoproteins was observed with tristearin and triolein. Lymph and hepatic cholesterol, and lymph triglycerides were highest with safflower oil, suggesting endogenous mobilization. Cholesterol production was least with triolein. Sterol synthesis was greatest with tristearin, perhaps attributable to decreased negative feedback analogous to effects of cholestyramine. Differences in lipoprotein composition observed with the various diets are important since effects on particle size and shape may influence removal mechanisms. The mechanisms underlying the different effects of dietary triglycerides on sterol absorption and metabolism remain to be elucidated.

**UPTAKE AND ESTERIFICATION OF CIRCULATING CARNITINE BY AORTA AND HEART IN RABBITS IN VIVO. INFLUENCE OF DIETARY CHOLESTEROL.** P.J. Gillies and F.P. Bell (Dept. of Pathology, Faculty of Med., McMaster Univ., Ontario, Canada) *Atherosclerosis* 33(1), 99-109 (1979). Disappearance of intravenously injected DL-[methyl- $^{14}C$ ] carnitine from the bloodstream and its uptake and esterification by heart and aorta were studied in rabbits fed atherogenic or non-atherogenic control diets. The disappearance rate of [ $^{14}C$ ] carnitine from the bloodstream was approximately 2-fold greater in animals fed the control diet than in those fed the atherogenic diets. Analysis of defined arterial segments indicated that aortas in animals fed the atherogenic diet contained greater [ $^{14}C$ ] carnitine activity (4- to 8-fold) and greater acetyl- [ $^{14}C$ ] carnitine activity (4-fold) when compared to aortas of control animals; uptake of plasma [ $^{14}C$ ] carnitine and accumulation of acyl- [ $^{14}C$ ] carnitine compounds by the heart was independent of diet. Butyryl- [ $^{14}C$ ] carnitine, although not detected in aortas from animals fed the non-atherogenic or atherogenic diet for only 7 weeks, was detected in aortas from animals fed the atherogenic diet 17 weeks.

**LOW DENSITY LIPOPROTEIN RECEPTOR ACTIVITY ON SKIN FIBROBLASTS FROM RHESUS MONKEYS WITH DIET-INDUCED OR SPONTANEOUS HYPERCHOLESTEROLEMIA.** L.A. Guertler and R.W. St. Clair (Dept. of Pathology and Arteriosclerosis Research Center, Bowman Gray School of Med., Winston-Salem, NC 27103) *J. Biol. Chem.* 255(1), 92-9 (1980). The purpose of this study was to evaluate low density lipoprotein (LDL) receptor activity on skin fibroblasts from rhesus monkeys with either spontaneous or diet-induced hypercholesterolemia in order to determine whether a defect in LDL receptor function was associated with the hyperbetalipoproteinemia in either of these conditions. Spontaneous hypercholesterolemia, in the two rhesus monkeys studied here, even though having many of the phenotypic characteristics of familial hypercholesterolemia in human beings, was not associated with a similar defect in LDL receptor function.

**REGULATION OF CHOLESTEROL SYNTHESIS IN PRIMARY RAT HEPATOCYTE CULTURE CELLS. POSSIBLE REGULATORY SITE AT STEROL DEMETHYLATION.** C. Havel, E. Hansbury, T.J. Scallen, and J.A. Watson (Dept. of Biochem. and Biophys., and The Liver Center, Univ. of California, San Francisco, CA 94143) *J. Biol. Chem.* 254(19), 9573-82 (1979). Primary rat hepatocyte culture cells were used to study the acute regulation of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase activity in response to 25-hydroxycholesterol, 3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -cholestantriol, and mevalonolactone. All three effectors caused a rapid suppression of HMG-CoA reductase activity. 25-Hydroxycholesterol also caused an increase in the ratio of newly synthesized methyl sterols to newly synthesized C $_{27}$ -sterols. The rats of both unesterified and esterified sterol synthesis increased as a function of exogenous mevalonolactone concentration. However, there was a direct relationship between the accumulation of methyl sterols and the decrease in HMG-CoA reductase activity. The results described in the present article support an important and perhaps necessary relationship between the rate of methyl sterol conversion of C $_{27}$ -sterols and the suppression or inhibition of HMG-CoA reductase in primary hepatocyte culture cells.

**CONTROL OF LIVER LIPID ACCUMULATION IN LAYING BIRDS.** L.S. Jensen (Dept. of Poultry Sci., Univ. of Georgia, Athens, Georgia 30602) *Fed. Proc.* 38(120) 2631-4 (1979). There is accelerated liver lipid metabolism associated with ova development. Dietary components, nutritive and nonnutritive, are known to influence liver lipid synthesis and secretion. Choline may not be synthesized at a rate fast enough to meet the needs of laying hens for prevention of fatty livers and is definitely inadequately synthesized in Japanese quail. A fatty liver-hemorrhagic syndrome (FLHS) is observed in caged laying hens fed a corn base diet that appears to be nutritionally adequate. However, supplementation of isoenergetic, equifat, isonitrogenous diets with small quantities of feed-

stuffs such as fish meal or brewer's yeast prevents the abnormal liver lipid accumulation and associated hemorrhages. Higher circulating estrogen levels appear to be associated with the disease. A similar condition can be reproduced in growing birds injected with  $\beta$ -estradiol-17-dipropionate. These fatty livers result from accelerated lipogenesis. Prevention of FLHS by brewer's yeast and certain other common feedstuffs appears to be due to a nutrient complex. Selenium and/or vitamin E is needed in combination with an unidentified factor(s) to facilitate normal lipid metabolism in the laying hen. Adding selenium and/or Vitamin E alone has little or no effect on liver lipid accumulation unless added in combination with a source of the unidentified factor. An understanding of the mechanism of action of this nutrient complex must await the identification of the unknown component(s).

**SEQUESTRATION AND EXCRETION OF HIGH DENSITY AND LOW DENSITY LIPOPROTEINS BY THE KIDNEY IN HUMAN NEPHROTIC SYNDROME.** M.L. Kashyap, B.S. Ooi, B.A. Hynd, C.J. Glueck, V.E. Pollak, and K. Robinson (Lipid Res. and Nephrology Divs., Dept. of Med. and the General Clin. Res. Center, Univ. of Cincinnati College of Med., Cincinnati, OH 45267) *Artery* (Leonidas, MI) 6(2):108-21 (1979). Using immunofluorescence, double immunodiffusion, and lipoprotein electrophoresis urinary lipoproteins were studied in 9 subjects with nephrotic syndrome, and renal (biopsy) tissue lipoproteins in 5 of them. High density lipoproteins were detected in the urine of all nephrotic patients; none were present in urine of normal controls or subjects with primary hyperlipoproteinemia and normal renal function. Low density lipoproteins were detected in 2 of the 9 nephrotic subjects, and in none of the controls or subjects with primary hyperlipoproteinemia. High and low density lipoprotein antigens were detected in renal tissue of all 5 nephrotic subjects having tissue available for analysis. The data suggest that the nephrotic kidney may sequester and excrete high and low density lipoproteins, and lend support to the possibility that abnormal urinary loss of circulating lipoproteins may contribute to accelerated catabolism of high and low density lipoproteins in nephrotic syndrome.

**PROSTAGLANDINS AND THEIR PRECURSORS IN TISSUES FROM RATS FED ON TRANS,TRANS-LINOLEATE.** J.E. Kinsella, D.H. Hwang, P. Yu, J. Mai and J. Shimp (Dept. of Food Science, Cornell Univ., Ithaca, NY 14853) *Biochem. J.* 184(3), 701-4 (1979). Feeding *trans,trans*-9,12-linoleate to rats at 50 and 100% of the dietary fat decreased the concentrations of *n*-6 fatty acids, i.e. 18:2, 20:3 and 20:4, in heart, kidney, lung, adipose tissue and platelets of rats. The concentrations of prostaglandin products prostaglandins E<sub>1</sub>, E<sub>2</sub> and F<sub>2 $\alpha$</sub>  in serum were significantly decreased in rats receiving high concentrations of dietary *trans,trans*-linoleate.

**ATHEROGENIC DIETS AND NEUTRAL-LIPID ORGANIZATION IN PLASMA LOW DENSITY LIPOPROTEINS.** T. Kirchhausen, S.H. Untracht, G.M. Fless, and S.M. Scanu (Dept. of Med., Biophys. and Theoretical Biol., Biochem., The Univ. of Chicago, Pritzker Schl. of Med., and The Franklin McLean Memorial Res. Inst., Chicago, IL 60637 (U.S.A.)) *Atherosclerosis* 33(1), 59-70 (1979). The plasma low density lipoproteins (LDL) of rhesus monkeys fed 3 atherogenic diets exhibited thermal transitions at temperatures much higher (37-43C) than those observed in control animals or in normal humans (20-33 C). The same differences were noted in the neutral lipids (cholesteryl esters and triglycerides) which were isolated from the respective lipoproteins. In particular, the difference in thermal properties between the normal and abnormal LDL's was attributable to subtle differences in their cholesteryl ester composition (mainly an increase in the saturated and monosaturated fatty acid moieties), with altered triglyceride contents playing only a minor role. Thus, at body temperature, the hyperlipidemia that follows the administration of atherogenic diets is associated with a high degree of order of the neutral lipids in the core of the LDL particle. This, in turn, may be related to the atherogenicity of the abnormal lipoprotein species.

**ABSENCE OF FATTY LIVERS IN RHESUS MONKEYS FED OROTIC ACID.** M. Korycka-Dahl, T. Richardson, C.H. Amundson and J.R. Allen (Dept. of Food Sci., Univ. of Wisconsin, Madison WI 53706) *J. Dairy Sci.* 62(11), 1801-3 (1979). Pairs of rhesus monkeys were fed for 10 wk a basal diet containing 1% orotic acid or 10% nonfat milk powders. Amounts of total lipids in the liver and hepatic morphology were normal after 10 wk indicating that orotic acid in the diet did not induce fatty livers in rhesus monkeys.

**INCREASED BINDING OF LOW DENSITY LIPOPROTEIN TO LIVER MEMBRANES FROM RATS TREATED WITH 17 $\alpha$ -ETHINYL ESTRADIOL.** P.T. Kovanen, M.S. Brown, and J.L. Goldstein (Depts. of Molecular Genetics and Internal Med., Univ. of Texas Health Sci. Center at Dallas, Dallas, TX 75235) *J. Biol. Chem.* 254(22), 11367-73 (1979). Pharmacologic doses of 17 $\alpha$ -

ethinyl estradiol have been reported to cause a marked lowering of plasma lipoprotein levels in the rat. The drop in plasma low density lipoprotein (LDL) is associated with enhanced uptake of LDL by the liver. In the current studies, we show that membranes prepared from livers of ethinyl estradiol-treated rats exhibit a 3- to 10-fold increase in saturable binding sites for human <sup>125</sup>I-LDL. These binding sites resembled the LDL receptors previously described in extrahepatic human, mouse, and bovine cells in that they: 1) showed a marked preference for human LDL as opposed to human high density lipoprotein (HDL); 2) required calcium; 3) failed to bind LDL in which the lysine residues had been acetylated or methylated *in vitro*; and 4) were destroyed by pronase.

**EFFECT OF HYDRAZINE EXPOSURE ON HEPATIC TRIACYLGLYCEROL BIOSYNTHESIS.** R.G. Lamb and W.L. Banks, Jr. (Depts. of Pharmacology, Med., Biochem. and the MCV Cancer Center, Med. College of Virginia, Richmond, VA 23298) *Biochim. Biophys. Acta* 574(3), 440-7 (1979). Acute hydrazine exposure elevated rat liver triacylglycerol content and produced a rapid rise in triacylglycerol production from *sn*-[1,3-<sup>14</sup>C]glycerol 3-phosphate by liver homogenate and microsomal fractions. Hydrazine treatment also increased the incorporation of [1,3-<sup>14</sup>C]glycerol into hepatic triacylglycerol by the intact animal. Homogenates of hepatocyte monolayers exposed to hydrazine *in vitro* also exhibited an increased capacity to form triacylglycerol from *sn*-[1,3-<sup>14</sup>C]glycerol 3-phosphate. Hydrazine-dependent increases in hepatic triacylglycerol production measured *in vitro* correlated well with an increase in microsomal phosphatidate phosphohydrolase (EC 3.1.3.4) activity. Therefore, the fatty liver associated with hydrazine exposure may be explained in part by a rise in the enzymatic capacity of hepatic triacylglycerol biosynthesis.

**LONG-TERM EFFECT OF THE COMBINATION OF CALCIUM CLOFIBRATE AND CALCIUM CARBONATE ON SERUM TOTAL CHOLESTEROL, TRIGLYCERIDE AND HIGH DENSITY LIPOPROTEIN-CHOLESTEROL CONCENTRATIONS ON HYPERLIPOPROTEINAEMIA. A COMPARATIVE STUDY WITH CLOFIBRATE.** A. Lehtonen and J. Viikari (Dept. of Med., Univ. Central Hospital of Turku, 20520 Turku, Finland) *Atherosclerosis* 33(1), 49-58 (1979). Thirty hyperlipidaemic patients (19 with type IIA, 4 with IIB and 7 with type IV hyperlipoproteinaemia) were subjected to therapy with calcium clofibrate and calcium carbonate (4C, 2 + 2 g/day for 6 months) and the effect was compared with clofibrate (1C, 2 g/day) which was given for 6 months as well, in a single-blind placebo-controlled study. 4C and 1C decreased total serum cholesterol levels especially in subgroups IIA and IIB. 4C was somewhat more effective than 1C in decreasing (VLDL + LDL)-cholesterol in subgroup IIA. The HDL-cholesterol concentrations and the ratio of HDL-cholesterol and total cholesterol increased during treatment with both 1C and 4C. The HDL-cholesterol increase (vs. placebo) was 18%. The concentrations of serum triglycerides decreased by 33% during both treatment periods and there was no significant difference between 1C and 4C.

**THE EFFECT OF SIALIC ACID REMOVAL ON VERY LOW DENSITY LIPOPROTEIN.** C.L. Malmendier, W.W. Feremans and M. Fontaine (Res. Unit of Atherosclerosis, Lab. of Chem. Pathology and Clin. Chem., St. Pierre Univ. Hosp. and Lab. of Pathology and Electron Microscopy, Free Univ. of Brussels, Brussels Belgium) *Artery* (Leonidas, MI) 6(2), 144-56 (1979). All procedures such as incubation, dialysis, and concentration used in the preparation of desialyzed lipoproteins, reduced the triglyceride and cholesterol concentrations of human very low density lipoproteins (VLDL). The neuraminidase preparation used contained no lipolytic activity but some proteolytic activity. Sialic acid removal on VLDL modified the particle's electrophoretic mobility on agarose and the migration of apoproteins C-III<sub>2</sub> and C-III<sub>1</sub> to a C-III<sub>0</sub> mobility on PAGE. Apo C-III<sub>1</sub> appeared more resistant to enzyme action. Sialic acid removal from VLDL also caused the formation of aggregates of 8000 Å as demonstrated by electron microscopy, and as suggested by the increase in turbidity. The above modifications may be responsible for the changes in catabolism of desialyzed lipoproteins reported in animals. This report represents preliminary data leading to subsequent studies on the turnover of desialyzed lipoproteins in man.

**REGULATION OF DE NOVO PHOSPHATIDYLCHOLINE SYNTHESIS IN RAT INTESTINE.** C.M. Mansbach, II, and S. Parthasarathy (Veterans Admin. Hosp. and Div. of Gastroenterology, Dept. of Med., Duke Univ. Med. Center, Durham, NC 27710) *J. Biol. Chem.* 254(19), 9688-94 (1979). The question of the step at which *de novo* phosphatidylcholine synthesis is regulated was studied in rats with common bile duct and duodenal cannulae. The rats were infused with a triacylglycerol emulsion, containing either 0 or 10 mM phosphatidylcholine, for 3 h. From 5 to 120 min prior to death, <sup>32</sup>P<sub>i</sub> was injected intraperitoneally. <sup>32</sup>P incorporation into phosphocholine, CDP-choline, and phosphatidylcholine was studied.

The data indicate that phosphocholinesterase is the most likely enzyme to be the primary regulatory enzyme in *de novo* phosphatidylcholine synthesis. This enzyme was studied in microsomal preparations from the intestine of similarly prepared rats. The permeability of the microsomes to glucose 6-phosphate and EDTA was found to be increased on phosphatidylcholine infusion as well as an increase in phosphatidylcholine and phosphatidylethanolamine content of the microsomes. It is postulated that alterations in the microsomal membrane affect phosphocholinesterase activity which regulates *de novo* phosphatidylcholine synthesis.

TRUE AND APPARENT METABOLIZABLE ENERGY VALUE OF FAT FOR LAYING HENS: INFLUENCE OF LEVEL OF USE. G.G. Mateos and J.L. Sell (Dept. of Animal Science, Iowa State Univ., Ames, Iowa 50011) *Poult. Sci.* 59(2), 369-74 (1980). White Leghorn hens in egg production were fed rations that contained 0, 3, 6, 9, 12, or 15% yellow grease. True metabolizable energy (TME) and nitrogen-corrected apparent metabolizable energy (AME<sub>n</sub>) values of the rations were determined, and these data were used to estimate the time and AME<sub>n</sub> of yellow grease. An inverse relationship was observed between the level of yellow grease supplementation and the TME and AME<sub>n</sub> values of this fat source. The TME and AME<sub>n</sub> of yellow grease were 11,567 and 9,367 kcal/kg, respectively, when the supplemental level was 3%. Corresponding values when yellow grease constituted 15% of the ration were 8,907 and 8,653 kcal/kg, respectively. Variation in fatty acid composition of total dietary fat and associated changes in the TME and AME<sub>n</sub> of yellow grease are discussed.

REDUCTION OF HEPATIC LIPID DEPOSITION IN LAYING HENS BY DIETARY SELENIUM-YEAST INTERACTION. D.V. Maurice and L.S. Jensen (Dept. of Poultry Sci., Univ. of Georgia, Athens, GA 30602) *Poult. Sci.* 58(6), 1548-56 (1979). Experiments were conducted to study the effect of chromium and selenium on liver lipid deposition and incidence of liver hemorrhage in caged layers. Commercial strains of layers were fed *ad libitum* equaloric and isonitrogenous diets. Corn-torula dried yeast diets containing added selenium (.1 µg/g) with or without supplementary chromium (10 µg/g) significantly reduced total liver lipid and liver hemorrhage. The effects of protein source (soybean meal vs yeast) and selenium were separated in a factorial experiment which showed that the hepatic lipid response to selenium results from an interaction of selenium with an unidentified factor in torula yeast. The addition of selenium to diets with each protein source significantly elevated glutathione peroxidase (GSHPx) activity. Inclusion of 5% brewers yeast in the corn-soy diet or vitamin E (50 IU/kg) to the corn-torula dried yeast reduced liver lipid similar to that seen in birds fed the torula-yeast diet containing .1 µg Se/g. Comparison of oral glucose tolerance of birds fed corn-soy and corn-soy brewers yeast diets showed no significant difference. None of the dietary treatments significantly altered body weight, egg production, egg weight, or feed consumption. The results indicate that the metabolic role of selenium in relation to its role in hepatic lipid metabolism is mediated through an interaction with a dietary factor(s) present in yeast.

THE INSULIN-LIKE EFFECT OF HYDROGEN PEROXIDE ON PATHWAYS OF LIPID SYNTHESIS IN RAT ADIPOCYTES. J.M. May and C. de Haën (Depts. of Med., Med. Coll. of Va., Richmond, VA 23298 and Univ. of Wash., Seattle, WA 98195) *J. Biol. Chem.* 254(18), 9017-21 (1979). In addition to the well known insulin-like effects of certain concentrations of H<sub>2</sub>O<sub>2</sub> on glucose transport and oxidation in isolated rat adipocytes, the present work demonstrates that lipid synthesis from glucose is also enhanced over a narrow range of H<sub>2</sub>O<sub>2</sub> concentrations (0.15 to 0.5 mM) added to the incubation medium. As in the case of insulin, H<sub>2</sub>O<sub>2</sub> was found to stimulate greater glucose incorporation into glyceride-fatty acid than incorporation into glyceride-glycerol. The findings add to the growing list of insulin effects that are reproduced by H<sub>2</sub>O<sub>2</sub>, and strengthen the hypothesis that assigns H<sub>2</sub>O<sub>2</sub> the role of "second messenger" of insulin.

THE INTERACTIVE EFFECT OF DIETARY GLYCEROL AND CORN OIL ON RAT LIVER LIPIDS, SERUM LIPIDS AND SERUM LIPOPROTEINS. K.A. Narayan and J.J. McMullen (Biochem. and Nutr. Group, Food Sciences Lab., U.S. Army Natick Res. and Dev. Command, Natick, Mass. 91760) *J. Nutr.* 109(11), 1836-46 (1979). The long-term effects of dietary glycerol in the absence and presence of dietary corn oil on rat tissue lipids and six density classes of serum lipoproteins were investigated in young male Holzman rats. The food consumption was lower in the glycerol, corn oil group than in the glucose, corn oil group. The weight gain was substantially less in fat-free groups A (glucose) and B (glycerol) compared with corn oil-containing groups C (glucose) and D (glycerol), but the glycerol treatment (glycerol, fat-free and glycerol, corn oil) resulted in increased liver mass, irrespective of whether there was fat in the diet or not. After 21 weeks, liver cholesterol and triglycerides increased between 1- and 2-fold in

group B (glycerol, fat-free) compared with group A (glucose, fat-free) and confirmed previous results with old rats from this laboratory. The serum chylomicra significantly increased in both glycerol groups but more so in group D (glycerol, corn oil) than in all other groups. These results have generally indicated a beneficial interactive effect of corn oil in glycerol-containing diets, but the mechanism of accelerated induction of fatty livers in rats fed fat-free diets containing glycerol remains to be explored further.

CHANGES IN CHOLESTEROL METABOLISM IN INFANTS IN RESPONSE TO DIETARY CHOLESTEROL AND FAT. P.J. Nestel, A. Poyser and T.J.C. Boulton (Cardiovascular Metabolism and Nutr. Res. Unit, Baker Med. Res. Inst., Melbourne, Australia) *Amer. J. Clin. Nutr.* 32(11), 2177-82 (1979). The regulation of the serum cholesterol level in infancy is not understood but it has been suggested that it is less precise than in adulthood. Ten infants, ages 3 to 16 months, were studied during two periods of 1 month each, first consuming a low-cholesterol, polyunsaturated fatty acid-rich diet and later a cholesterol containing, polyunsaturated fatty acid-poor diet. The mean observed and predicted differences in the serum cholesterol level were, respectively, 56 and 68 mg/100 ml suggesting that the magnitude of the response becomes established in early life. Bile acid excretion was significantly higher with dietary polyunsaturated fatty acids, probably explaining some of the effect on the serum cholesterol. The sterol balance data showed that the net sterol balance fell substantially during the consumption of cholesterol in seven of the 10 infants. Although a steady state for cholesterol metabolism is not being claimed for growing infants, the fall in the net sterol balance is strongly suggestive of lessened endogenous cholesterol synthesis as reported in adults.

UPTAKE AND DEGRADATION OF <sup>125</sup>I-LABELLED HIGH DENSITY LIPOPROTEINS IN RAT LIVER CELLS IN VIVO AND IN VITRO. L. Ose, T. Ose, K.R. Norum and T. Berg (Inst. for Nutr. Res., Schl. of Med., Univ. of Oslo, Blindern, Oslo, Norway) *Biochim. Biophys. Acta* 574(3), 521-36 (1979). The uptake of <sup>125</sup>I-labelled high density lipoproteins (HDL) in various organs of the rat was determined after an intravenous injection. The uptake of <sup>125</sup>I-labelled polyvinylpyrrolidone in the same organs was determined in order to assess uptake by fluid endocytosis. The uptake/organ was highest for the liver. The adrenals showed the highest uptake/unit weight of the organs studied. The liver, the kidneys and the spleen showed comparable values for uptake/g of tissue. The uptake of <sup>125</sup>I-labelled HDL exceeded by far that of <sup>125</sup>I-labelled polyvinylpyrrolidone in the liver, the kidneys, the spleen and the adrenals, indicating that the uptake of <sup>125</sup>I-labelled HDL was mediated by adsorptive endocytosis. The in vitro uptake and degradation of <sup>125</sup>I-labelled HDL in isolated rat hepatocytes was studied. Subcellular fractionation by isopycnic centrifugation indicated that the accumulation of <sup>125</sup>I-labelled HDL did not take place in the lysosomes, but rather on the plasma membrane and possibly in the endosomes (phagosomes). Chloroquine, but not the protease inhibitor leupeptin, reduced the hydrolysis of the cholesteryl ester moiety of HDL.

METABOLISM OF VERY LOW DENSITY LIPOPROTEINS BY HUMAN MONONUCLEAR CELLS. A. Poyser and P.J. Nestel (Baker Med. Res. Inst., Commercial Road, Melbourne, Victoria, 3181, Australia) *Artery* (Leonidas, MI) 6(2), 122-43 (1979). The binding characteristics and the uptake and degradation of human very low density lipoproteins (VLDL, d < 1.006) by cultured lymphocytes were determined. The rates of uptake and degradation of <sup>125</sup>I-VLDL (Sf 60-400) at 37 degrees C were greatest at concentrations below 50 µg VLDL protein/ml medium. To determine whether C as well as B apoproteins of VLDL were being catabolized, tetramethyl urea (TMU)-precipitable and TMU-soluble proteins were labelled so that their relative rates of metabolism could be compared by the ratios of the two isotopes. Incubations of lymphocytes with FLDL labelled in this manner demonstrated uptake and degradation of both TMU-precipitable and TMU-soluble proteins. VLDL isolated from hypertriglyceridaemic individuals were on average metabolized more rapidly than VLDL isolated from normolipidaemic individuals. Because of the heterogeneity of VLDL particles it is possible that VLDL from hypertriglyceridaemic subjects contain a higher proportion of particles (possibly remnants) with a greater affinity for cellular receptors.

ORIGIN OF CHOLESTEROL TRANSPORTED IN INTESTINAL LYMPH: STUDIES IN PATIENTS WITH FILARIAL CHYLURIA. A.C.R. Quintão, A. Drewiacki, K. Stechhahn, E.C. de Faria, and A.M. Sipahi (Dept. of Internal Med., Hospital das Clínicas, The Univ. of São Paulo Med. School, São Paulo, Brazil) *J. Lipid Res.* 20(8), 941-51 (1979). In subjects fed a cholesterol-free diet there are three possible sources of intestinal lymph cholesterol: a) mucosal synthesis; b) absorption of endogenous (biliary) cholesterol; and c) transudation of plasma lipoproteins into the lacteals of the

intestinal wall. To test these possibilities, the extent of transudation was measured by means of [ $^3\text{H}$ ]  $\beta$ -sitosterol administered intravenously as a marker. Absorption of biliary cholesterol was reduced by oral administration of  $\beta$ -sitosterol (9 g/day), and mucosal synthesis of cholesterol was evaluated by comparisons of plasma/lymph [ $^{14}\text{C}$ ] cholesterol specific activity ratios after intravenous administration of a single dose of labeled cholesterol. Studies were carried out on six patients with filarial chyluria. In five patients fed a cholesterol-free diet the results indicated that lymph cholesterol was largely derived by transudation of plasma lipoproteins into the lacteals from the intestinal blood supply, without contribution from de novo mucosal synthesis or from absorption of endogenous cholesterol. The intestinal lymph of one patient fed cholesterol (2 g/day) contained cholesterol originating mostly from plasma transudation and from dietary absorption, with little contribution from absorbed endogenous cholesterol.

## Fats and oils

ANALYSIS OF POLYGLYCEROLS AND OTHER POLYOLS FROM EMULSIFIERS BY HPLC. K. Aitzetmüller, et al. *Fette, Seifen, Anstrichm.* 81(11), 436-41 (1979). Polyglycerols and other polyols can be conveniently analyzed by partition HPLC on silica columns. Derivatization is not necessary. Compounds of this type are often found in the esterified form in food emulsifiers, and are set free upon saponification. They are also used as humectants and freezing point depressants, e.g. in soft ice cream. A chemical classification of food emulsifiers is given, and the identification of their neutral polyols by HPLC is described.

WINTERIZATION OF SUNFLOWER OIL. W. Kehse. *Fette, Seifen, Anstrichm.* 81(12), 463-6 (1979). Properties of sunflower oil that influence the process of winterization were investigated as were the conditions of crystallization of waxes and mixed chain glycerides. Filtration performance and the significance of the use of filter aids are discussed in examples of described plants. A new type of filter appears promising for the solution of problems associated with winterization.

GEOMETRICAL AND POSITIONAL ISOMER CONTENT OF THE MONOUNSATURATED FATTY ACIDS FROM VARIOUS RAT TISSUES. R. Wood, R. Chumbler, M. Matocha, and A. Zoeller (Dept. of Biochem. and Biophys., Texas Agr. Exp. Station, Texas A&M Univ. System, College Station, TX 77843) *Lipids* 14(9), 789-94 (1979). The percentage distribution of the geometrical and positional isomers in the hexadecenoates and octadecenoates isolated from triglycerides, phosphatidylcholines, and phosphatidylethanolamines of tissues from normal rats maintained on a laboratory diet has been determined. Generally, palmitoleic was the predominant hexadecenoate, but many of the tissue phospholipids contained relatively high percentages of the  $\Delta 6$  and  $\Delta 7$  isomers. These data add to our basic information about the percentage distribution of geometrical and positional isomers of naturally occurring unsaturated fatty acids in the major lipid classes of various normal tissues. Some new concepts were advanced as possible explanations to some of the observed positional isomer distributions.

A SIMPLIFIED PROCEDURE FOR THE DETERMINATION OF BETAININE IN LIVER. A.J. Barak and D.J. Tuma (Liver Study Unit, Veterans Administration Hospital and the Departments of Internal Medicine and Biochemistry, University of Nebraska Medical Center, Omaha, NE) *Lipids* 14(10), 860-3 (1979). A convenient procedure for the determination of hepatic betaine levels is described. The method takes advantage of ethanol precipitation to rid acidified tissue extracts of interfering substances. Betaine is reacted with potassium triiodide to form betaine periodide, which is selectively precipitated via pH adjustment. The precipitate of betaine periodide is dissolved in ethylene dichloride and measured spectrophotometrically. The method is specific, accurate, and simple and showed recoveries of from 97 to 103% at two different levels of added betaine. The applicability of the method was shown when it was demonstrated that diets containing different amounts of choline influenced levels of hepatic betaine.

PROTON AND CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDIES OF RHODOPSIN-PHOSPHOLIPID INTERACTIONS. N. Zumbulyadis and D.F. O'Brien (Res. Laboratories, Eastman Kodak Co., Rochester, NY 14650) *Biochemistry* 18(24): 5427-5432 (1979). Proton and carbon-13 nuclear magnetic resonance ( $^1\text{H}$  and  $^{13}\text{C}$  NMR) spectra of rhodopsin-phospholipid membrane vesicles and sonicated disk membranes are presented and discussed. The presence of rhodopsin in egg phosphatidylcholine

vesicles results in homogeneous broadening of the methylene and methyl resonances. This effect is enhanced with increasing rhodopsin content and decreased by increasing temperature. The proton NMR data indicate the phospholipid molecules exchange rapidly ( $< 10^{-3}$  s) between the bulk membrane lipid and the lipid in the immediate proximity of the rhodopsin. These interactions result in a reduction in either or both the frequency and amplitude of the tilting motion of the acyl chains. The  $^{13}\text{C}$  NMR spectra identify the acyl chains and the glycerol backbone as the major sites of protein lipid interaction. In the disk membranes the saturated *sn*-1 acyl chain is significantly more strongly immobilized than the polyunsaturated *sn*-2 chain. This suggests a membrane model in which the lipid molecules preferentially solvate the protein with the *sn*-1 chain, which we term an edge-on orientation. The NMR data on rhodopsin-asclectin membrane vesicles demonstrate that the lipid composition is not altered during reconstitution of the membranes from purified rhodopsin and lipids in detergent.

ISOLATION OF PRECURSORS OF MUTTON ODOR. E. Wong and A.F. Mabrouk (Dept. of Scientific & Industrial Research, Private Bag, Palmerston North, New Zealand) *J. Agric. Food Chem.* 27(6), 1415-6 (1979). Water-soluble constituents of lean mutton have been fractionated and odor significant fractions have been subjected to systematic separation by a combination of adsorption, ion exchange, and paper chromatographic steps, with sensory assessment of fractions being carried out concomitantly. A single fraction has been obtained which on heating to 180 C produced odor notes reminiscent of cooked mutton.

ESTIMATION OF OXIDATIVE DETERIORATION OF OILS AND FOODS BY MEASUREMENT OF ULTRAWEAK CHEMILUMINESCENCE. R. Usuki, T. Kaneda, A. Yamagishi, C. Takyu and H. Inaba (Dept. of Food Chem., Faculty of Agr., Tohoku Univ., Sendai 980, Japan) *J. Food Sci.* 44(6), 1573-6 (1979). The present study was undertaken to evaluate the oxidative deterioration of oils and foods by using the single photoelectron counting system, which was designed for the measurement of ultraweak chemiluminescence. Thermally oxidized soybean oils were prepared and their chemical characteristics and chemiluminescence were measured. The results showed that the increase of emission intensity was closely correlated to the oxidative deterioration of oils, instant Chinese noodles and milk powder. This new method has a great advantage in measuring the quality of food in a short period of time by a nonexpert without any prior treatment.

FATTY ALCOHOLS IN CAPELIN, HERRING AND MACKEREL OILS AND MUSCLE LIPIDS: II. A COMPARISON OF FATTY ACIDS FROM WAX ESTERS WITH THOSE OF TRIGLYCERIDES. W.N. Ratnayake and R.G. Ackman (Chem. Dept., Dalhousie Univ., Halifax N.S. B3H 4J3, Canada) *Lipids* 14(9), 804-10 (1979). The fatty acids recovered from the triglycerides and wax esters of common northwest Atlantic copepods are compared with the fatty acids of wax esters recovered intact from certain fish skin and body lipid, and from commercial fish oils. The fish species, herring, capelin and mackerel, all feed on copepods, and many resemblances of the copepod lipid fatty acids to those of a previous analysis of similar copepods suggest that the basic dietary fat input for these fish may be quite constant. The two copepod fatty acid analyses differed quantitatively in triglyceride 20:1 and 22:1 and also in 20:5 $\omega$ 3 and 22:6 $\omega$ 3, confirming the primary role of the wax esters in copepods. Selectivity factors are discussed in comparing the copepod wax ester fatty acids with the fatty acids of the wax esters recovered intact from the fish lipids and oils. The basic role of copepods in supplying all types of fatty acids to fish depot fats is considered to be strongly supported by these findings.

DISTINCT EFFECTS OF THREE BILE SALTS ON CHOLESTEROL SOLUBILIZATION BY OLEATE-MONOOLEIN-BILE SALT MICELLES. J.C. Montet, M.O. Reynier, A.M. Montet and A. Gerolami (Unité de Recherches de Pathologie Digestive, U 31 INSERM, 46, Boulevard de la Gaye, 13009 Marseille, France) *Biochim. Biophys. Acta* 575(2), 289-94 (1979). Micellar cholesterol solubilities in bile salt-monoolein-oleic acid systems have been determined. Whatever the bile salt/oleyl compounds ratio, taurochenodeoxycholate solubilizes more cholesterol than taurocholate and much more than taurooursodeoxycholate. At pH 6.7, the cholesterol solubility limit is about the same with either oleate or monoolein. Cholesterol solubility falls in oleate-bile acid mixtures as the pH is raised. The capacity for supersaturation with cholesterol is greater for bile salt-monoolein than for bile salt-oleate micells. For the latter it decreases as pH increases.

CHANGES IN LIPID COMPOSITION OF COOKED MINCED CARP (*CYPRINUS CARPIO*) DURING FROZEN STORAGE. J. Mai and J.E. Kinsella (Dept. of Food Sci., Inst. of Food Sci., Cornell Univ., Ithaca, NY 14853) *J. Food Sci.* 44(6), 1619-24



(1979). Minced carp tissue was cooked by baking and deep-fat frying and stored at -18 C for periods up to 8 wk. Phospholipid (PL) levels decreased whereas free fatty acids (FFA) increased during frozen storage of all samples. Samples treated with antioxidants gave significantly ( $P < 0.05$ ) higher values for FFA compared to the controls. Thiobarbituric acid (TBA) values were higher in the cooked samples compared to the raw samples. Samples without antioxidants had considerably higher TBA values than those containing antioxidants. The carbonyl content of the samples fluctuated during storage. There was no significant change in the composition of the fatty acids during storage.

A NEW SERIES OF LONG-CHAIN DICARBOXYLIC ACIDS WITH VICINAL DIMETHYL BRANCHING FOUND AS MAJOR COMPONENTS OF THE LIPIDS OF *BUTYRIVIBRIO* SPP. R.A. Klein, G.P. Hazelwood, P. Kemp and R.M.C. Dawson (M.R.C. Biochem. Parasitology Unit, Molteno Inst., Univ. of Cambridge, Cambridge CB2 3EE, U.K.) *Biochem. J.* 183(3), 691-700 (1979). Some members of the genus *Butyrivibrio*, including a general fatty acid auxotroph (strain S2), contain as a major part of their complex lipids a high-molecular-weight component that is probably formed by the union of two fatty acid chains [Hazelwood & Dawson (1979) *J. Gen. Microbiol.* 112, 15-27]. Proton and  $^{13}\text{C}$  n.m.r. and i.r. and mass spectroscopy were used to examine a homologous series of these moieties and, in addition, the hydrocarbon derivative of one

homologue and several synthetic compounds. The results indicate that the high-molecular-weight components are a series of long-chain dicarboxylic acids containing vicinal dimethyl branching, located near the centre of the chain.

CONTENT AND STABILITY OF  $\alpha$ -TOCOPHEROL IN FRESH AND DEHYDRATED PEPPER FRUITS (*CAPSIDUM ANNUUM* L.). J. Kanner, S. Harel and H. Mendel (Div. of Food Technology, Institute for Technology & Storage of Agricultural Products, Agricultural Research Organization, Volcani Center, Bet Dagan 50-100, Israel) *J. Agric. Food Chem.* 27(6), 1316-8 (1979). The content of  $\alpha$ -tocopherol in pepper fruits (*Capsicum annum* L.) and its stability during dehydration and storage were determined. Our data show that the three pepper varieties used in this study, regardless of stage of maturity, contained from 9000 to 10000  $\mu\text{g}$  of  $\alpha$ -tocopherol/g of oil (oleoresin). The  $\alpha$ -tocopherol content in the fresh pepper and its dry matter was found to depend on the content of lipids which in turn depends on ripening stage and genetic variety factors. During dehydration the loss of  $\alpha$ -tocopherol in red pepper fruits was less than 5%. The  $\alpha$ -tocopherol was found to be unstable in powdered pepper stored at low water activity,  $a_w$ , but very stable at high  $a_w$ . The large amount of  $\alpha$ -tocopherol found in the fresh ripe fruits, ca. 3-10 mg/100 g, indicates that this vegetable could become an important source of vitamin E in the human diet.

LACK OF REGIOSELECTIVITY IN FORMATION OF OXOHYDROXYOCTADECENOIC ACIDS FROM THE 9- OR 13-HYDROPEROXIDE OF LINOLEIC ACID. H.W. Gardner and R. Kleiman (Northern Regional Res. Center, U.S. Dept. of Agr., Peoria, Illinois 61604) *Lipids* 14(10), 848-51 (1979). Either 9-hydroperoxy-*trans*-10, *cis*-12-octadecadienoic acid or 13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid was treated with the catalyst, cysteine- $\text{FeCl}_3$ , in the presence of oxygen. Oxohydroxyoctadecenoic acids were among the many products formed as a result of hydroperoxide decomposition. A mixture of 9(13)-oxo-13(9)-hydroxy-*trans*-11(10)-octadecenoic acids ( $\delta$ -ketols) was produced from either isomeric hydroperoxide. The formation of isomeric  $\delta$ -ketols from 9-hydroxy-*trans*-12,13-epoxy-*trans*-10-octadecenoic acid (epoxyol), a known product of 13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid decomposition, implies that the epoxyol is an intermediate. The mechanism was elucidated by the facile conversion of the epoxyol (methyl ester) to methyl 9(13)-oxo-13(9)-hydroxy-*trans*-11(10)-octadecenoates with a Lewis acid,  $\text{BF}_3$  etherate.

THE SOLID STATE ACYL SHIFT OF DIGLYCERIDES: AN ELECTRON DIFFRACTION STUDY. D.L. Dorset and W.A. Pangborn (Medical Foundation of Buffalo, Inc., 73 High St., Buffalo, NY 14203) *Chem. Phys. Lipids* 25(2), 179-89 (1979). The progress of the solid state acyl shift of 1,2-diglycerides to 1,3-diglycerides is followed at room temperature in single dipalmitin microcrystals by electron diffraction. The  $\beta'$  form, rather than the  $\alpha$ -form of the 1,2-isomer, transforms to the 1,3 product. The  $\beta'$  form packs in the monoclinic paraffin fashion, i.e. the  $\text{O}_1$  methylene subcell and a chain tilt of 27 degrees about the long subcell axis. After the isomerization, the chain tilt (14 degrees to surface normal) occurs around the  $b_2^*$  axis of the resultant  $\text{T}_{11}$  methylene subcell.

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**SYNTHESIS AND CHARACTERIZATION OF [1-<sup>13</sup>C]- AND D<sub>8</sub>-ARACHIDONIC ACID.** Un Hoi Do, M.G. Sundaram, S. Ramachandran, and R.W. Bryant (Applied Science Division, Milton Roy Company, State College, Pennsylvania 16801) *Lipids* 14(9), 819-21 (1979). Methyl d<sub>8</sub>- and [1-<sup>13</sup>C] 5,8,11,14-eicosatetraenoate (arachidonate) were prepared from a common synthetic precursor, 4,7,10,13-nonadecatetrayn-1-ol. The purified products were characterized by gas chromatography-mass spectrometry. Mass spectra of *t*-butyldimethylsilyl esters of d<sub>8</sub>- and [1-<sup>13</sup>C]-arachidonic acid showed a most intense [M-57]<sup>+</sup> peak at high mass. The isotopic purity of methyl [1-<sup>13</sup>C] arachidonate was 99% and that of methyl d<sub>8</sub>-arachidonate was 56%. When d<sub>8</sub>-arachidonic acid was prepared by direct deuteration of 5,8,11,14-eicosatetraenoic acid, the isotopic purity of the sample was 86%.

**QUANTITATIVE ANALYSIS OF MONOSIALOGLANGLIOSIDES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF THEIR PERBENZOYL DERIVATIVES.** E.G. Bremer, S.K. Gross and R.H. McCluer (Eunice Kennedy Shriver Center, Waltham MA 92154) *J. Lipid Res.* 20(8), 1028-35 (1979). A quantitative high-performance liquid chromatographic method for the analysis of monosialogangliosides as their perbenzoyl derivatives has been devised. To take advantage of the high sensitivity of the HPLC, a small-scale isolation method for gangliosides was devised. This method coupled with HPLC isotope dilution analysis was used to analyze the G<sub>M3</sub> content of 1 ml of human plasma.

## Drying oils and paints

**COATINGS BASED ON FATTY ACID MODIFIED CELLULOSE PHTHALATE RESINS.** B.P. Singh, S. Chandra and A.K. Vasishtha (Department of Oil and Paint Technology, Harcourt Butler Technological Institute, Kanpur 208002, India) *J. Oil Colour Chem. Assoc.* 62, 470-4 (1979). Cellulose mixed esters of phthalic acid and long chain fatty acids were prepared and their film properties were studied and compared with those of corresponding alkyds. The fatty acid modified cellulose phthalate coatings exhibited excellent physical, chemical and mechanical properties and were found to be superior to the corresponding alkyds.

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