American Oil Chemist's Society

62nd

ANNUAL MEETING

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PROGRAM

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MAY 2-6, 1971

Shamrock Hilton Hotel

Houston, Texas

MONDAY MORNING—MAY 3

10:00 A.M.—Camellia Room

SESSION A—WAX ESTER METABOLISM

Chairman—J. C. Nevenzel, University of California, Los Angeles

10:00 INTRODUCTORY REMARKS

10:05 1. **WAX ESTER METABOLISM IN FISH ROE**
D. M. Sand, J. L. Hehl and Hermann Schlenk, The Hormel Institute

10:25 2. **ENZYMATIC SYNTHESIS OF LONG CHAIN ALCOHOLS AND WAX ESTERS FROM FATTY ACIDS**
P. E. Kolattukudy, Washington State University

10:45 3. **THE BIOSYNTHESIS OF WAX ESTERS: ENZYMIC INTERCONVERSIONS OF FATTY ACIDS AND FATTY ALCOHOLS**
Fred Snyder and Boyd Malone, Oak Ridge Associated Universities

11:05 4. **UNUSUAL WAX ESTERS FROM PORPOISE ACOUSTIC AND NONACOUSTIC TISSUES**
Usha Varanasi and D. C. Malins, National Marine Fisheries Service

11:25 5. **WAX ESTER LIPASES OF MARINE ANIMALS**
Elizabeth A. Baker, J. C. Nevenzel and A. A. Benson, Scripps Institute of Oceanography

11:45 6. **WAX ESTER METABOLISM DURING THE DEVELOPMENT OF COPEPODS**
R. F. Lee, Scripps Institute of Oceanography, Jed Hirota, J. C. Nevenzel, A. R. Lewis, A. A. Benson

MONDAY MORNING—MAY 3

10:00 A.M.—Bluebonnet Room

SESSION B—CHEMISTRY

Chairman—G. D. Lichtenwalter, Armour Industrial Chemical Co.

10:00 INTRODUCTORY REMARKS

10:10 7. **SYNTHESIS AND CHARACTERIZATION OF 9,10-METHYLENE-10-OCTADECENE, AN ANALOGUE OF STERCULENE**
James Reed Clark and Henry Kircher, University of Arizona

10:30 8. **THERMOGRAVIMETRIC OBSERVATIONS ON THE FORMATION OF Δ^2-OXAZOLINES FROM β-HYDROXYALKYLAMIDES**
W. E. Parker, T. A. Foglia, G. Maerker, Eastern Regional Research Lab.

10:50 9. **EVIDENCE FOR ADDITION COMPOUNDS OF OXIDIZED TOCOPHEROL AND SOYBEAN LECITHIN**
W. L. Porter, A. S. Henick and L. A. Levasseur, U.S. Army Natick Labs.

11:10 10. **RUBBER SWELL AS A FUNCTION OF FATTY ACID ESTER CHAIN LENGTH**
A. Eisner, R. E. Koos, A. Bilyk, W. E. Parker, G. Maerker, Eastern Regional Research Lab.

11:30 11. **FUTURE TRENDS IN COMMERCIAL HYDROGENATION OF TRIGLYCERIDE OILS**
L. F. Albright, Purdue University

MONDAY MORNING—MAY 3

10:00 A.M.—Azalea Room

SESSION C—EDIBLE OILS

Chairman—J. C. Cowan, Northern Regional Research Lab.

10:00 INTRODUCTORY REMARKS

10:10 12. **TERTIARY-BUTYLHYDROQUINONE TREATMENT OF CRUDE SUNFLOWERSEED OIL**
B. M. Luckadoo, E. R. Sherwin, Eastman Chemical Products, Inc.

10:30 13. **CRAMBE SEED PROCESSING: DECOMPOSITION OF THIOGLUCOSIDES WITH CHEMICAL ADDITIVES**
L. D. Kirk, G. C. Mustakas, A. N. Booth, E. L. Griffin, Jr., Northern Regional Research Lab.

10:50 14. **COMMERCIALIZED CONTINUOUS HYDROGENATION YIELDS MAXIMUM PROFITS AND PRODUCT FLEXIBILITY**
R. A. Coombes, R. A. Zavada, Blaw-Knox Chemical Co.

11:10 15. **ORGANOLEPTIC AND OXIDATIVE STABILITY OF BLENDS OF SOYBEAN AND PEANUT OILS**
J. C. Cowan, Helen A. Moser, G. R. List and C. D. Evans, Northern Regional Research Lab.

11:30 DISCUSSION PERIOD

MONDAY MORNING—MAY 3

10:00 A.M.—Continental Room

SESSION D—MYCOTOXINS

Chairman—M. E. Whitten, ARS, USDA

10:00 INTRODUCTORY REMARKS

10:10 16. **DETECTION OF AFLATOXIN USING SMALL COLUMNS OF FLORISIL**
James Velasco, ARS, USDA

10:30 17. **USE OF GLC IN DETECTING THE PRESENCE OF ACTIVELY METABOLIZING ASPERGILLUS PARASITICUS IN PEANUT STOCKS**
Louise S. Lee, Alva F. Cucullu, W. A. Pons, Jr., Southern Regional Research Lab.

10:50 18. **COTTONSEED STORAGE: EFFECT OF TEMPERATURE AND MOISTURE ON INCREASE OF AFLATOXIN CONTENT**
M. E. Whitten, L. L. Smith, ARS, USDA

11:10 19. **STUDIES ON TWO TOXIC LIPIDS PRODUCED BY ASPERGILLUS FLAVUS**
A. A. Adekunle and O. Bassir, University of Idaban, Nigeria

MONDAY MORNING—MAY 3

10:00 A.M.—Grecian Room

SESSION E—ANALYTICAL PROCEDURES: I.

Chairman—R. O. Feuge, Southern Regional Research Lab.

10:00 20. **AUTOMATIC REDUCTION OF SPECTROPHOTOMETER DATA BY SMALL COMPUTERS: APPLICATION TO THIN LAYER CHROMATOGRAPHIC ANALYSIS OF PHOSPHOLIPIDS**
G. G. Nelson, Lawrence Radiation Laboratory

10:20 21. **A RAPID PROCEDURE FOR DETERMINATION OF RESIDUAL HEXANE IN OILSEED FLOUR**
Sara P. Fore and H. P. Dupuy, Southern Regional Research Lab.

10:40 22. **ANALYSIS OF VEGETABLE OILS FOR RESIDUAL SOLVENT AND VOLATILES**
H. P. Dupuy and Sara P. Fore, Southern Regional Research Lab.

11:00 23. **A HYDROGEN BROMIDE TITRATION FOR SOAPS IN FAT PRODUCTS**
R. O. Feuge, Zigrida Zarins and J. L. White, Southern Regional Research Lab.

11:20 24. **A QUANTITATIVE TLC METHOD FOR THE ANALYSIS OF GERMICIDES IN PERSONAL CARE PRODUCTS**
I. I. Domsky and M. B. Graber, Armour-Dial, Inc.

11:40 25. **ANALYSIS FOR PHOSPHATE IN DETERGENTS**
J. C. Abbott, P. H. Garrison, Procter & Gamble Co.

MONDAY AFTERNOON—MAY 3

2:00 P.M.—Camellia Room

SESSION F—ANALYSIS AND BIOSYNTHESIS OF GLYCEROLIPIDS: I.

Chairman—Carter Litchfield, Rutgers University

2:00 26. **SYNTHESIS OF PHOSPHOGLYCERIDES**
R. G. Jensen, University of Connecticut

2:40 27. **ISOMERIZATION OF L-GLYCERALDEHYDE TO DIHYDROXYACETONE PHOSPHATE DURING GLYCERIDE SYNTHESIS**

G. Ananda Rao, M. F. Sorrels and R. Reiser, Texas A&M University

3:00 28. **BIOSYNTHESIS OF PHOSPHATIDYL GLYCEROL CELL-FREE EXTRACTS OF SPINACH LEAVES**
Morris Kates and M. O. Marshall, University of Ottawa

3:20 29. **CHOLINE DEFICIENCY AND THE METABOLISM OF PHOSPHATIDYLETHANOLAMINE**
R. Lyman, G. Sheehan and R. Babcock, University of California

3:40 30. **GLYCOLIPID METABOLISM IN TUMORIGENIC VIRUS-TRANSFORMED CELL LINES**
R. O. Brady, P. T. Mora, F. A. Cumar, National Institutes of Health

4:00 31. **SILVER NITRATE THIN LAYER CHROMATOGRAPHY OF PHOSPHOLIPIDS. SEPARATION OF PIG AND SHRIMP PHOSPHATIDYLCHOLINES AND PHOSPHATIDYLETHANOLAMINES**
J. M. Shaw and N. R. Bottino, Texas A&M University

4:20 32. **POSITIONAL DISTRIBUTION OF THE FATTY ACIDS IN THE PHOSPHOLIPIDS OF BOVINE RETINA ROD OUTER SEGMENTS**
R. E. Anderson and Linda Sperling, Baylor College of Medicine

MONDAY AFTERNOON—MAY 3

2:00 P.M.—Bluebonnet Room

SESSION G—SYMPOSIUM: LIPOPIGMENTS: I. BIOGENESIS AND CHEMICAL COMPOSITION

Chairman—**A. N. Siakotos**, Indiana University Medical Center

2:00 33. **INTRODUCTORY REMARKS**
Fredrick Bernheim, Duke University Medical Center

2:10 34. **CHEMICAL REACTION MECHANISMS IN LIPID PEROXIDATION**
R. J. Horvat, R. B. Russell Research Center

2:40 35. **TOXICITY OF LIPID PEROXIDES**
Fredrick Bernheim, Duke University Medical Center

3:10 36. **LIPID PEROXIDATION IN MODEL MEMBRANE SYSTEMS**
Albert Barber, Lynn S. Grinna, David Meyer and H. M. Tinberg, University of California

3:40 37. **LIPID OXIDATION IN BIOLOGICAL MEMBRANES**
Y. Hatefi and W. G. Hanstein, Scripps Clinic and Research Foundation

4:10 38. **EVIDENCE FOR FREE-RADICAL DAMAGE TO MEMBRANE PHOSPHOLIPIDS BY OXIDATIVE ENZYMES**
P. R. McCay, J. L. Poyer and P. M. Pfeifer, Oklahoma Medical Research Foundation

4:40 39. **THE ROLE OF PEROXIDATION IN THE INDUCTION OF HEPATIC INJURY**
N. R. DiLuzio, Tulane University Medical School

MONDAY AFTERNOON—MAY 3

2:00 P.M.—Azalea Room

SESSION H—RECENT ADVANCES IN DRUGS AFFECTING LIPID METABOLISM

Chairman—**M. G. Horning**, Baylor College of Medicine

2:00 40. **EFFECTS OF ANDROGENS ON SERUM LIPIDS LIPOPROTEINS**
Antal Solyom, National Institutes of Health

2:45 41. **ESTROGEN EFFECT AND LIPID METABOLISM**
R. H. Furman, Eli Lilly Co.

3:30 42. **PROGESTINS, ANABOLIC-ANDROGENS, ESTROGENS: EFFECTS ON TRIGLYCERIDES AND POSTHEPARIN LIPOLYTIC ACTIVITIES**
C. J. Glueck, D. Scheel, J. Fishback and P. Steiner, University of Cincinnati

4:15 43. **INHIBITION OF HEPATIC TRIGLYCERIDE BIOSYNTHESIS BY CLOFIBRATE**
H. J. Fallon, L. L. Adams and R. G. Lamb, University of North Carolina

MONDAY AFTERNOON—MAY 3

2:00 P.M.—Continental Room

SESSION I—THE PLANT GENETICIST'S CONTRIBUTION TOWARDS CHANGING THE LIPID AND AMINO ACID COMPOSITION OF OILSEEDS

Chairman—**G. C. Cavanagh**, Ranchers Cotton Oil

2:00 **INTRODUCTORY REMARKS**

2:05 44. **IMPROVING HIGH-ERUCIC OILSEEDS: CHEMICALLY OR GENETICALLY?**
W. H. Tallent, Northern Regional Research Lab.

2:25 45. **SESAME SEED**
D. M. Yermanos, University of California

2:45 46. **THE PLANT GENETICIST'S CONTRIBUTION TOWARDS CHANGING THE LIPID AND AMINO ACID COMPOSITION OF COTTONSEED**
R. J. Miravalle, National Cottonseed Products, Inc.

3:05 47. **THE PLANT GENETICIST'S CONTRIBUTION TOWARD CHANGING THE LIPID AND AMINO ACID COMPOSITION OF OILSEEDS: SAFFLOWER**
Paul Knowles, University of California

3:25 48. **PROBLEMS IN BREEDING ALTERED LIPID AND AMINO ACID COMPOSITION OF SUNFLOWER SEED**
Murray Kinmam, ARS, USDA

3:45 49. **BREEDING FOR IMPROVED FATTY ACID CONTENT OF SOYBEAN OIL**
R. W. Howell and C. A. Brim, Crops and Research Division, ARS, USDA

4:05 50. **IMPROVING SOYBEAN OIL STABILITY BY PLANT BREEDING**
E. G. Hammond, W. R. Fehr, H. E. Snyder, Iowa State University

4:25 **PANEL DISCUSSION**

MONDAY AFTERNOON—MAY 3

2:00 P.M.—Grecian Room

SESSION J—ENVIRONMENTAL ASPECTS OF OIL AND OILSEED PROCESSING: I.

Chairman—**J. C. Dietz**, Clark Dietz & Associates

2:00 **INTRODUCTION**

2:15 51. **STATE AND FEDERAL REGULATORY ASPECTS OF ENVIRONMENTAL CONTROL**
C. W. Klassen, Environmental Consultant, Springfield, Illinois

3:00 52. **IN PLANT CONTROL OF WASTE WATERS**
J. F. Byrd and G. N. McDermott, Procter & Gamble Co.

3:45 53. **OIL SEPARATION: THEORY AND PRACTICE**
W. J. Katz, Rex-Chainbelt, Inc.

4:30 54. **JOINT TREATMENT OF INDUSTRIAL AND MUNICIPAL WASTES**
A. F. Vondrick, City of Phoenix, Arizona

TUESDAY MORNING—MAY 4

9:00 A.M.—Camellia Room

SESSION K—ANALYSIS AND BIOSYNTHESIS OF GLYCEROLIPIDS: II.

Chairman—**R. G. Jensen**, University of Connecticut

9:00 55. **STEREOSPECIFIC ANALYSIS OF TRIGLYCERIDES**
Hans Brockerhoff, Fisheries Research Board of Canada

9:40 **56. SYNTHESIS OF STEREOSPECIFICALLY SUBSTITUTED MONO-, DI- AND TRIACYLGLYCEROLS**
Dmytro Buchnea, University of Toronto

10:00 **57. METABOLIC BASIS OF NONRANDOM STRUCTURE OF NATURAL TRIGLYCERIDES**
A. Kuksis, W. C. Breckenridge and B. J. Holub, University of Toronto

10:20 **58. STEREOSPECIFIC ANALYSES OF TRIGLYCERIDES FROM SEED OILS OF LIMANTHES DOUGLASII AND MONNINA EMARGINATA**
B. E. Phillips and C. R. Smith, Jr., Northern Regional Research Lab.

10:40 **59. GLYCERYL ETHERS IN INSECTS: BIOSYNTHESIS OF ALKYL AND ALK-1-ENYL ETHANOLAMINE PHOSPHOGLYCERIDES**
E. N. Lambremont, Louisiana State University

11:00 **60. TUMOR LIPIDS: CARBON NUMBER DISTRIBUTION OF TRIGLYCERIDES AND GLYCERYL ETHER**
Randall Wood, V.A. Hospital

11:20 **61. COMPARATIVE LIPID PATTERNS IN TWO FRESHWATER DOLPHINS, INIA GEOFFRENSIS AND SOTALIA FLUVIATILUS**
Carter Litchfield, Rutgers University, John Kinneman, R. G. Ackman, C. A. Eaton

TUESDAY MORNING—MAY 4

9:00 A.M.—Bluebonnet Room

SESSION L—SYMPOSIUM: LIPOPIGMENTS: II. BIOGENESIS AND CHEMICAL COMPOSITION

Chairman—**A. N. Siakotos,** Indiana University Medical Center

9:00 **62. THE FINE STRUCTURE OF LIPOPIGMENTS**
Itaru Watanabe, Indiana University Medical Center

9:30 **63. ISOLATION TECHNIQUES AND LIPID COMPOSITION OF LIPOPIGMENTS**
A. N. Siakotos, Indiana University Medical Center

10:00 **64. ENZYMES ASSOCIATED WITH LIPOPIGMENTS**
V. Patel, Indiana University Medical Center

10:30 **65. CATION COMPOSITION IN LIPOPIGMENTS**
C. Childress and A. N. Siakotos, University of Kansas School of Medicine

11:00 **66. THE CURRENT STATUS OF MELANIN AND MELANOSOMES**
Angelo Cantaboni, Indiana University Medical Center

11:30 **67. DISORDERS OF LIPID PEROXIDATION—A VIDEO TAPED CLINICAL CONFERENCE OF NEURONAL CEROID LIPOFUSCINOSIS**
W. Zeman, Indiana University Medical Center

TUESDAY MORNING—MAY 4

9:00 A.M.—Azalea Room

SESSION M—RECENT ADVANCES IN DRUGS AFFECTING LIPID METABOLISM: II.

Chairman—**R. H. Furman,** Eli Lilly Co.

9:00 **68. EFFECTS OF ETHYL-p-CHLOROPHENOXY-ISOBUTYRATE (CIPB) ON BILIARY SECRETION**
G. L. Jordan, Jr., E. C. Horning and M. G. Horning, Baylor Medical School

9:45 **69. ORAL CONTRACEPTIVES AND LIPID METABOLISM IN THE RAT**
Lilla Aftergood and Roslyn B. Alfin-Slater, University of California

10:30 **70. CLINICAL EVALUATION OF MK-185: A NEW HYOLIPIDEMIC DRUG**
C. R. Sirtori and D. L. Azarnoff, University of Kansas Medical Center

11:15 **71. EVALUATIVE STUDIES OF THE HYPOLIPIDEMIC AGENT SaH 42-348 IN ANIMALS AND MAN**
L. A. Kelly, A. R. Timms, J. H. Trapold, R. T. Graupner and J. J. Cillo, Sandoz Pharmaceuticals

TUESDAY MORNING—MAY 4

9:00 A.M.—Continental Room

SESSION N—PORTER WILLIAMS SYMPOSIUM ON PROTEINS

Chairman—**R. A. Phelps,** Anderson, Clayton & Co.

9:00 **INTRODUCTORY REMARKS**

9:10 **72. THE SOCIO-ECONOMICS OF IMPROVING NUTRITION: ROLE OF GOVERNMENT AND INDUSTRY**
Aaron Altschul and Graham Williams, U.S. Department of Agriculture

10:10 **73. SOME CONSEQUENCES OF THE USE OF LINEAR PROGRAMMING TECHNIQUES FORMULATING HUMAN DIETS**
H. L. Wilcke, D. T. Hopkins and L. W. DeClue, Ralston-Purina

11:10 **74. THE INTERRELATIONSHIP OF CHEMICAL STRUCTURE AND ENVIRONMENT TO FUNCTIONALITY OF PROTEINS**
Wilda H. Martinez, ARS, USDA

TUESDAY MORNING—MAY 4

10:00 A.M.—Grecian Room

SESSION O—ENVIRONMENTAL ASPECTS OF OIL AND OILSEED PROCESSING: II.

Chairman: **John E. Heilman,** Allied Mills, Inc., Chicago

10:00 **75. NEW CONCEPTS IN DEFUSED AERATION**
Walter Zentner, Walker Process Equipment Co.

10:30 **76. OXYGEN USE IN THE ACTIVATED SLUDGE PROCESS**
J. R. McWhirter, Union Carbide Corp.

11:00 **77. CONTROL OF POLLUTANTS FROM AIR AND WATER FOR THE OILSEEDS INDUSTRY**
K. W. Becker, Blaw Knox Chemical Plants, Inc.

11:30 **78. AIR POLLUTION—MANPOWER AND TRAINING NEEDS**
H. P. Kramer, National Air Pollution Control Administration

TUESDAY AFTERNOON—MAY 4

2:00 P.M.—Camellia Room

SESSION P—ANALYSIS AND BIOSYNTHESIS OF GLYCEROLIPIDS: III.

Chairman—**G. A. Rao,** Texas A&M University

2:00 **79. DIFFERENTIAL SCANNING CALORIMETRY OF SINGLE ACID TRIGLYCERIDES: EFFECT OF CHAIN LENGTH AND UNSATURATION**
J. W. Hagemann, W. H. Tallent, Northern Regional Research Lab., K. E. Kolb

2:20 **80. THERMAL PROPERTIES OF 2-OLEODIPALMITIN AND 2-ELAIDODIPALMITIN AND SOME OF THEIR MIXTURES AS DETERMINED BY DIFFERENTIAL SCANNING CALORIMETRY**
N. V. Lovegren, M. S. Gray, R. O. Feuge, Southern Regional Research Lab.

2:40 **81. CONSTITUENTS OF ECHINACEA ANGUSTIFOLIA ROOTS WITH INSECT JUVENILIZING ACTIVITY**
Martin Jacobson, R. E. Redfern, USDA, Agricultural Research Center, USDA

3:00 **82. LIPID COMPOSITION OF SUBCELLULAR PARTICLES OF PUPAE OF THE BOLL WEEVIL, ANTHONOMUS GRANDIS BOH**
A. C. Thompson, R. D. Henson, R. C. Gueldner, P. A. Hendin, Boll Weevil Research Lab.

3:20 **83. LIPIDS OF THE AVOCADO FRUIT COAT (PERSEA GRATISSIMA): LIPID COMPOSITION AND THE COMPONENT TRIGLYCERIDES**
C. B. Sharma, Glenda Martinez, Marshall University

TUESDAY AFTERNOON—MAY 4

2:00 P.M.—Bluebonnet Room

SESSION Q—PROSTAGLANDINS

Chairman—**A. J. Vergroesen**, Unilever Research Laboratories, Vlaardingen/Duiven

2:00 INTRODUCTORY REMARKS

2:05 84. THE DISTRIBUTION, BIOSYNTHESIS AND RELEASE OF RENAL PROSTAGLANDINS
Keith Crowshaw, Saint Louis University Medical School

2:35 85. PROSTAGLANDIN BIOSYNTHESIS WITH SPECIAL REFERENCE TO ADIPOSE TISSUE
E. J. Christ, Unilever Research Laboratories, Vlaardingen/Duiven

3:05 86. THE EFFECT OF PROSTAGLANDIN E_1 AND LINOLEIC ACID ON EXPERIMENTAL ARTERIAL THROMBOSIS IN RATS
G. Hornstra, Unilever Research Laboratories, Vlaardingen/Duiven

3:35 87. RELATIONS BETWEEN PROSTAGLANDINS AND CARBOHYDRATE METABOLISM
B. May, Medizinische Hochschule, Hannover, Germany

4:05 88. PROSTAGLANDIN ANTAGONISM
J. H. Sanner, Searle & Co.

4:35 89. THE EFFECTS OF PROSTAGLANDINS ON THE HYPODYNAMIC FROG HEART COMPARED WITH THOSE OF FATTY ACIDS, EPINEPHRINE AND ADENOSINE PHOSPHATES
A. J. Vergroesen and J. de Boer, Unilever Research Laboratories, Vlaardingen/Duiven

5:05 89a. PROSTAGLANDIN BIOSYNTHESIS IN THE HUMAN PLACENTA
P. T. Russell, University of Cincinnati College of Medicine

TUESDAY AFTERNOON—MAY 4

2:00 P.M.—Azalea Room

SESSION R—SYMPOSIUM: CHEMICAL MODIFICATIONS

Chairman—**Edwin Frankel**, Northern Regional Research Lab.

2:00 INTRODUCTORY REMARKS

2:05 90. CRAFT-POLYMER SHRINK-PROOF TREATMENTS OF WOOL FROM OLEIC SAFFLOWER FATTY ACIDS
M. G. Diamond, Carl Ellinger, Glenn Fuller and E. N. Frankel, Western Regional Research Lab.

2:35 91. N,N-DISUBSTITUTED FATTY AMIDES AS PLASTICIZERS AND PLASTICIZER-STABILIZERS FOR POLYVINYL CHLORIDE RESINS
F. C. Magne, R. R. Mod and Gene Sumrell, Southern Regional Research Lab.

3:05 92. METHYL 9(10)-CARBOXYSTEARATE BY CATALYTIC AIR OXIDATION OF HYDROFORMYLATED OLEATE
A. W. Schwab, E. N. Frankel and J. C. Cowan, Northern Regional Research Lab.

3:35 93. SELECTIVE HYDROFORMYLATION OF POLYUNSATURATED FATS WITH A RHODIUM-TRIPHENYLPHOSPHINE CATALYST
E. N. Frankel and F. L. Thomas, Northern Regional Research Lab.

4:05 94. NEW POLYACETAL AND POLY(ESTER-ACETAL) COATINGS FROM HYDROFORMYLATED LINSEED OIL
T. H. Khoe, L. E. Gast, E. N. Frankel and J. C. Cowan, Northern Regional Research Lab.

TUESDAY AFTERNOON—MAY 4

2:00 P.M.—Continental Room

SESSION S—PORTER WILLIAMS SYMPOSIUM ON PROTEINS: II.

Chairman—**R. A. Phelps**, Anderson, Clayton & Co

2:00 95. FUNCTIONALITY AND END USE OF PROTEINS
R. E. Morse, Rutgers University

3:00 96. MODIFYING PROTEINS FOR MAXIMUM UTILIZATION IN THE RUMINANT
A. D. McGilliard, Iowa State University

4:00 97. PROTEINS FROM HYDROCARBONS: ECONOMIC POTENTIAL
C. F. Feldman, Gulf Oil Corp.

TUESDAY AFTERNOON—MAY 4

2:00 P.M.—Grecian Room

SESSION T—CHOLESTERYL ESTERS: I.

Chairmen—**K. K. Carroll**, University of Western Ontario, and **B. L. Walker**, University of Guelph

2:00 INTRODUCTORY REMARKS

2:10 98. RAT ADRENAL CHOLESTEROL ESTERS
B. L. Walker and J. A. Carney, University of Guelph

2:40 99. REGULATION OF OVARIAN CHOLESTEROL ESTERS BY GONADOTROPINS
H. R. Behrman, Harvard Medical School

3:00 100. COMPOSITION AND METABOLISM OF MILK CHOLESTEROL ESTERS
T. W. Keenan, Purdue University and Stuart Patton

3:40 101. FATTY ACID SPECIFICITY OF CHOLESTERYL ESTER HYDROLASE FROM RAT LIVER
Demetrios Sgoutas, Emory University

4:00 102. METABOLISM OF CHOLESTERYL ARACHIDONATE IN THE RAT
B. J. Holub and A. Kuksis, University of Toronto

4:40 103. EFFECTS OF DIET ON ESTERIFICATION AND OXIDATION OF 26-^{14}C-CHOLESTEROL IN RABBITS
K. K. Carroll and Djaja Suria, University of Western Ontario

WEDNESDAY MORNING—MAY 5

9:00 A.M.—Camellia Room

SESSION U—LIPID METABOLISM IN CELLS IN CULTURE: I. USE OF CELLS IN CULTURE TO STUDY METABOLIC RELATIONSHIPS

Chairman—**J. F. Mead, University of California**

9:00 INTRODUCTORY REMARKS

9:10 104. INSULIN AND DEXAMETHASONE EFFECTS ON RAT LIVER CELLS IN CULTURE
L. E. Gerschenson, University of California

9:40 105. LIPID ACCUMULATION IN MAMMALIAN CELLS
C. G. Mackenzie, Julia B. Mackenzie and O. K. Reiss, University of Colorado School of Medicine

10:10 106. STUDIES ON THE BIOSTABILITY OF TISSUE CULTURE CELL LIPIDS
R. P. Geyer, Harvard University

10:40 107. CELLULAR CONTROL MECHANISMS FOR LIPID TRANSPORT AND METABOLISM
J. M. Bailey, George Washington Medical School

11:10 108. CONVERSION OF CARBOHYDRATE INTO LIPIDS IN MALIGNANT AND NONMALIGNANT CULTURED LIVER CELLS
J. A. Watson, University of California Medical Center

11:40 GENERAL DISCUSSION

WEDNESDAY MORNING—MAY 5

9:00 A.M.—Bluebonnet Room

SESSION V—DETERGENTS

Chairman—**J. K. Weil**, Eastern Regional Research Lab.

9:00 INTRODUCTORY REMARKS

9:05 109. **THE EFFECTS OF ALKALIES ON POLYESTER**
J. J. Cramer, W. Lozo, J. D. Ciko, Wyandotte Chemicals Corp.

9:25 110. **DEVELOPMENT OF A PHOSPHATE FREE HOME LAUNDRY DETERGENT**
W. M. Linfield, K. A. Roseman and H. G. Reilich, IIT Research Institute

9:45 111. **EFFECT OF WATER HARDNESS AND DETERGENT BUILDERS ON ENZYME ACTIVITY**
W. L. Groves, B. W. Terry, R. D. Katstra, Continental Oil Co.

10:05 112. **SYNERGISTIC INTERACTIONS OF SHAMPOO INGREDIENTS**
P. M. Hay, Sandoz-Wander, Inc.

10:25 113. **TALLOW ALKANOLAMIDES: PREPARATION AND EFFECT ON SURFACTANT SOLUTIONS**
J. K. Weil, N. Parris, W. R. Noble, F. D. Smith, A. J. Stirton, Eastern Regional Research Lab.

10:45 114. **BIODEGRADATION OF SULFATED ALKANOLAMIDES**
T. C. Cordon, E. W. Maurer, A. J. Stirton, Eastern Regional Research Lab.

11:05 115. **SOME NEW BIODEGRADABLE SURFACTANTS DERIVED FROM CORN STARCH**
P. E. Throckmorton, David Aelony, R. R. Egan, Ashland Chemical Co., and F. H. Otey

WEDNESDAY MORNING—MAY 5

9:00 A.M.—Azalea Room

SESSION W—SULFOLIPIDS

Chairman—**Thomas Haines,** City University of New York

9:00 **INTRODUCTORY REMARKS**

9:10 116. **THE AZURE A METHOD FOR ANALYSIS OF SULFATIDES AND THE INFLUENCE OF THE STATE OF VITAMIN A DEFICIENCY ON THE FORMATION OF THIS SULFOLIPID, IN VIVO**
E. L. Kean, Case-Western Reserve University

9:40 117. **UNGULIC ACID AND ITS OCCURRENCE IN ANIMALS**
Erkki Leikola, University of Helsinki

10:10 118. **THE HALOSULFOLIPIDS OF OCHROMONAS DANICA**
T. H. Haines, City University of New York, Manuel Pousada, Alex Bruckstein, Bhupesh Das

10:40 119. **STRUCTURE OF A GLYCOLIPID SULFATE IN HALOBACTERIUM CUTIRUBRUM**
Morris Kates and Paul Deroo, University of Ottawa

11:10 120. **MYCOBACTERIAL SULFOLIPIDS**
M. B. Goren, National Jewish Hospital

WEDNESDAY MORNING—MAY 5

9:00 A.M.—Continental Room

SESSION X—FATTY ACID METABOLISM

Chairman—**P. K. Raju,** Texas A&M University

9:00 121. **SUBCELLULAR LOCALIZATION OF FATTY ACID SYNTHESIS IN SEEDS**
J. L. Harwood, P. K. Stumpf, University of California

9:20 122. **STIMULATION OF STEAROYL DESATURASE BY SN-GLYCERO-3-PHOSPHATE IN MOUSE LIVER MICROSOMES**
P. K. Raju and Raymond Reiser, Texas A&M University

9:40 123. **THE EFFECT OF NITRATE AND PHOSPHATE CONCENTRATIONS ON THE FATTY ACID METABOLISM OF THE ALGAE CHLAMYDOMONAS REINHARDTIS**
J. B. Saddler, J. B. Fagan, Frieda B. Taub, University of Washington

10:00 124. **FATTY ACIDS OF MOSS AND FERN LIPIDS**
W. H. Anderson, J. L. Gellerman and H. Schlenk, Hormel Institute

10:20 125. **DISCRIMINATION OF FATTY ACID ISOMERS BY THE LAYING HEN**
T. L. Mounts, E. A. Emken, W. K. Rohwedder and H. J. Dutton, Northern Regional Research Lab.

10:40 126. **VARIATIONS IN THE LIPID CONTENT DURING THE METAMORPHOSIS OF ANTHONOMUS GRANDIS (COLEOPTERA)**
R. D. Henson, A. C. Thompson, R. C. Gueldner, P. A. Hedin, Boll Weevil Research Lab.

11:00 127. **EFFECT OF MATURITY ON THE FATTY ACID COMPOSITION OF EIGHT VARIETIES OF PEANUTS GROWN AT PERKINS, OKLAHOMA IN 1968**
C. T. Young, Georgia Experiment Station, M. E. Mason, R. S. Matlock and G. R. Waller

11:20 128. **THE EFFECT OF ETHIONINE ON UNSATURATED FATTY ACID SYNTHESIS IN THE LIVER**
L. A. Witting, Elgin State Hospital

WEDNESDAY MORNING—MAY 5

9:00 A.M.—Grecian Room

SESSION Y—CHOLESTERYL ESTERS: II.

Chairman—David Kritchevsky, Wistar Institute, and W. Insull, Jr.

9:00 129. **THE CHOLESTEROL-RICH VERY LOW DENSITY LIPOPROTEINS OF BROAD BETA DISEASE (TYPE III HYPERLIPOPROTEINEMIA): RELATIONSHIP BETWEEN THE ABNORMALITIES OF LIPID COMPOSITION AND ELECTROPHORETIC MOBILITY**
W. R. Hazzard and E. L. Bierman, V. A. Hospital

9:30 130. **CHOLESTEROL ESTERS AND THE LESIONS OF ARTERIOSCLEROSIS IN MAN**
William Insull, Jr., Hahnemann Medical College, P. Dieter Lang, Yoshiya Hata, John Hower

10:00 131. **CHOLESTERYL ESTER METABOLISM IN NORMAL AND ATHEROSCLEROTIC ARTERIAL TISSUE**
R. W. St. Clair and H. B. Lofland, Jr., Bowman Gray School of Medicine

10:30 132. **CHOLESTERYL ESTER METABOLISM IN ARTERIAL TISSUE AND ATHEROMATA**
Seymour Dayton, V.A. Hospital, Los Angeles

11:00 133. **CHOLESTERYL ESTERS: THEORETICAL REFLECTIONS**
David Kritchevsky, Wistar Institute, and W. Insull, Jr.

11:30 134. **4-^{14}C-CHOLESTEROL DISTRIBUTION IN TISSUES OF RATS FED CORN OIL, BUTTERFAT AND CORN OIL, OR BUTTERFAT PLUS CHOLESTEROL DIETS**
Anahid Crecelius and R. B. Alfin-Slater, University of California, Los Angeles

WEDNESDAY AFTERNOON—MAY 5

2:00 P.M.—Camellia Room

SESSION Z—LIPID METABOLISM IN CELLS IN CULTURE: II. METABOLISM OF SPECIFIC LIPIDS AS STUDIED IN CELLS IN CULTURE

Chairman—**R. P. Geyer,** Harvard University

2:00 135. **THE METABOLISM OF FATTY ACIDS AND FATTY ALCOHOLS IN FIBROBLASTS (L-M CELLS) GROWN AS MONOLAYERS**
M. L. Blank, E. A. Cress and Fred Snyder, Oak Ridge Associated Universities

2:30 136. **PHOSPHOLIPID METABOLISM IN CELLS IN CULTURE**
W. E. Cornatzer, S. S. Tsao and D. J. Rytter, University of North Dakota Medical School

3:00 137. **STEROL FLUX AND SYNTHESIS IN TISSUE CULTURE CELLS**
G. H. Rothblat, Wistar Institute

3:30 138. **GLYCOSPHINGOLIPID METABOLISM IN FIBROBLASTS CULTURED FROM PATIENTS WITH INBORN ERRORS OF METABOLISM**
Glyn Dawson and Reuben Matalon, University of Chicago

4:00 **GENERAL DISCUSSION**

WEDNESDAY AFTERNOON—MAY 5

2:00 P.M.—Bluebonnet Room

SESSION AA—SYMPOSIUM: ENZYMES RELATED TO LIPOPROTEIN METABOLISM: I. LIPOPROTEIN LIPASE (LIPASES) AND HEPARIN ACTIVATED PHOSPHOLIPASE

Chairman—**R. I. Levy,** National Heart Institute, and **B. W. Shore,** Lawrence Radiation Laboratory

2:00 INTRODUCTORY REMARKS

2:10 139. PEPTIDE ACTIVATION OF FAT EMULSIONS FOR INTERACTION WITH LIPOPROTEIN LIPASE
R. J. Havel, San Francisco Medical Center

2:40 140. ROLE OF HDL PROTEIN IN LIPOPROTEIN LIPASE ACTIVITY
C. J. Fielding, University of Chicago

3:10 141. THE ROLE OF HEPARIN AS A POSSIBLE ALLOSTERIC MODIFIER OF LIPOPROTEIN LIPASE
J. M. Felts, University of Toronto, and T. F. Whayne, Jr.

3:40 142. CLINICAL AND BIOCHEMICAL DIFFERENTIATION OF POSTHEPARIN LIPOLYTIC ACTIVITY
R. M. Krauss, John LaRosa, Peter Herbert, R. I. Levy and D. S. Fredrickson, National Heart and Lung Institute

4:10 143. COMPARISON OF POSTHEPARIN LIPOLYTIC ACTIVITIES WITH TRIGLYCERIDE, MONOGLYCERIDE, AND PHOSPHOLIPID SUBSTRATES IN NORMAL AND HYPERTRIGLYCERIDEMIC SUBJECTS
W. C. Vogel, J. D. Brunzell and E. L. Bierman, V. A. Hospital

4:40 144. ACTIVATION OF LIPOPROTEIN LIPASE: AN EVALUTION OF CALCIUM AS A COFACTOR
T. F. Whayne, Jr., Ohio State University Medical College and J. M. Felts, University of Toronto

WEDNESDAY AFTERNOON—MAY 5

2:00 P.M.—Azalea Room

SESSION BB—MARKETING AND ECONOMICS

Chairman—**I. A. Wolff,** Eastern Regional Research Lab.

2:00 INTRODUCTORY REMARKS

2:05 145. COMMODITY PRICES AS RELATED TO VEGETABLE OIL MARKETING, PRICES AND UTILIZATION
D. M. Bartholomew, Merrill Lynch, Pierce, Fenner & Smith, Inc.

2:35 146. CONSUMPTION PATTERNS AND TRENDS FOR FATS AND OILS IN THE UNITED STATES
G. W. Kromer, U.S. Department of Agriculture

3:05 147. NEW MARKETS FOR TALLOW THROUGH RESEARCH
D. M. Doty, Fats & Protein Research Foundation, and W. J. Sheppard

3:35 148. ECONOMICS OF SUNFLOWER OIL PRODUCTION AND USE IN THE UNITED STATES
W. K. Trotter, W. D. Givan, R. B. Russell Agricultural Research Center

4:05 149. COST ANALYSES FOR NEW PRODUCTS AND PROCESSES DEVELOPED IN USDA LABORATORIES
V. E. Sohns, Northern Regional Research Lab.

WEDNESDAY AFTERNOON—MAY 5

2:00 P.M.—Continental Room

SESSION CC—SYMPOSIUM: LIPIDS IN REPRODUCTIVE TISSUE

Chairman—**J. G. Coniglio,** Vanderbilt University

2:00 INTRODUCTORY REMARKS

2:05 150. PATHWAYS OF PLACENTAL FATTY ACID METABOLISM
A. F. Robertson and Warren Karp, Ohio State University Hospitals

2:35 151. THE METABOLISM OF LIPIDS IN THE PLACENTA AND THE FETUS
J. F. Roux and Tamotsu Yoshioka, Cleveland General Metropolitan Hospital

3:05 152. LIPIDS IN HUMAN CERVICAL MUCUS
E. J. Singh and J. R. Swartout, University of Chicago

3:35 153. LIPID METABOLISM IN SPERMATOZOA
Charles Terner, Boston University

4:05 154. LIPID METABOLISM IN TESTIS
J. G. Coniglio, B. E. Evans and Richard Zseltvay, Vanderbilt University

4:35 QUESTIONS AND DISCUSSION

WEDNESDAY AFTERNOON—MAY 5

2:00 P.M.—Grecian Room

SESSION DD—ANALYTICAL PROCEDURES: II.

Chairman—**E. C. Horning,** Baylor Medical School

2:00 155. FATTY ACID POLYMORPHS: CORRELATION OF SOME IR, WIDE-LINE NMR, AND X-RAY DIFFRACTION PARAMETERS
A. V. Bailey, D. Mitcham, R. A. Pittman, Southern Regional Research Lab.

2:20 156. NEW GAS PHASE ANALYTICAL METHODS FOR THE STUDY OF STEROIDS
E. C. Horning, P. G. Devaus, N. Sakauchi, M. G. Horning, Baylor College of Medicine

2:40 157. ESTIMATION OF CIS/TRANS ISOMER CONTENT OF EDIBLE VEGETABLE OILS RAMAN SPECTROSCOPY
R. J. Horvat, R. B. Russell, Agricultural Research Center, G. F. Bailey

3:00 158. CONDUCTOMETRIC STUDIES OF ISOLATED NATURAL PHOSPHATIDYLCHOLINE IN THE ABSENCE AND PRESENCE OF STEROLS AND FAT SOLUBLE VITAMINS
J. T. Hoogeveen, V. A. Hospital

3:20 159. TOTAL SERUM LIPID DETERMINATION BY MEANS OF PULSATING NMR
H. Peeters and G. A. Persyn, Simon Steevin Institute, Brugge, Belgium

3:40 160. DEVELOPMENTS IN STATIONARY PHASES FOR GAS LIQUID CHROMATOGRAPHY ANALYSIS
D. M. Ottenstein, W. R. Supina, D. A. Bartley, Nicholas Pelick, Supelco, Inc.

4:00 161. ELEMENTAL C, H AND N ANALYSIS OF CRUSHED ROCK AND SOIL SAMPLES
F. T. Lindgren, G. R. Stevens, L. C. Jensen, University of California

4:20 162. A SIMPLIFIED HALPHEN PROCEDURE FOR CYCLOPROPENE FATTY ACIDS
E. C. Coleman and David Firestone, Food & Drug Administration

THURSDAY MORNING—MAY 6

9:00 A.M.—Camellia Room

SESSION EE—LIPID METABOLISM IN CELLS IN CULTURE: III. LIPID METABOLISM IN SPECIFIC CELLS AND THE USE OF CULTURED CELLS IN DIAGNOSIS OF GENETIC DISEASES

Chairman—**Roscoe Brady,** National Institutes of Health

9:00 163. CHARACTERISTICS OF CELLS DISSOCIATED FROM MOUSE MAMMARY GLANDS
S. Abraham, Children's Hospital Medical Center

9:30 164. LIPID METABOLISM IN BRAIN CELLS
J. H. Menkes, University of California

10:00 165. THE USE OF TISSUE CULTURE TECHNIQUES IN THE STUDY OF LIPID STORAGE DISEASES
H. R. Sloan, National Heart and Lung Institute

10:30 166. **THE USE OF CULTURED FETAL CELLS FOR THE ANTENATAL DIAGNOSIS OF LIPID STORAGE DISEASES**
Roscoe Brady, B. W. Uhlendorg, National Institute of Health, C. B. Jacobson, C. J. Epstein

11:00 **CONCLUSIONS AND DISCUSSIONS:** Moderator, David Kritchevsky, Wistar Institute

THURSDAY MORNING—MAY 6

9:00 A.M.—Bluebonnet Room

SESSION FF—SYMPOSIUM: ENZYMES RELATED TO LIPOPROTEIN METABOLISM: II. LECITHIN: CHOLESTEROL ACYLTRANSFERASE (LCAT) AND ITS RELATION TO SERUM LIPOPROTEINS

Chairmen—**J. A. Glomset,** University of Washington, and **A. V. Nichols,** University of California

9:00 **INTRODUCTORY REMARKS**

9:10 167. **LIPOPROTEIN ABNORMALITIES IN FAMILIAL LCAT DEFICIENCY**
K. R. Norum, John Glomset, University of Washington, A. V. Nichols and Trudy Forte

9:40 168. **STRUCTURE OF LIPOPROTEINS FROM LCAT DEFICIENT PLASMA**
Trudy Forte, University of California

10:05 169. **LCAT REACTIVITY AND SUBSTRATE INTERACTIONS**
A. V. Nichols, Walter Ho and Trudy Forte, University of California

10:30 170. **ON THE NATURE OF A LIPOPROTEIN (LP-X) CHARACTERIZING THE BILIARY OBSTRUCTION AND ITS POSSIBLE RELATIONSHIP TO LCAT**
P. Alaupovic, H. Magnani and D. Seidel, Oklahoma Medical Research Foundation

11:00 171. **EFFECTS ON LIPID PEROXIDE ON CHOLESTEROL ESTERIFICATION**
H. S. Mickel, E. L. Foulds, D. A. Clark, School of Aerospace Medicine

11:20 **CLOSING PANEL DISCUSSION:** Chairman—R. J. Havel
R. L. Levy, B. W. Shore, J. A. Glomset, A. V. Nichols, Pierre Alaupovic, E. L. Bierman

THURSDAY MORNING—MAY 6

9:00 A.M.—Azalea Room

SESSION GG—PLASMA AND MEMBRANE LIPOPROTEINS

Chairman—**H. Peeters,** Simon Steevin Institute, Brugge, Belgium

9:00 **INTRODUCTORY REMARKS**

9:05 172. **INFLUENCE OF APOPROTEINS ON LIPOPROTEIN STRUCTURE AND LIPID TRANSPORT**
A. M. Gotto, S. E. Lux, National Heart and Lung Institute

9:25 173. **DIFFERENTIATION OF LIPIDS IN PLASMA LIPOPROTEINS**
V. Blaton, Simon Steevin Institute, Brugge, Belgium

9:45 174. **COMPARATIVE STUDY OF LIPOPROTEINS IN PRIMATES**
H. Peeters, Simon Steevin Institute, Brugge, Belgium

10:05 175. **LIPID COMPOSITION AND PERMEABILITY OF MEMBRANES**
L. L. M. VanDeenen, University of Utrecht, The Netherlands

10:25 176. **PROTEIN-LIPID INTERACTION IN ERYTHROCYTE MEMBRANE**
R. F. A. Zwaal, University of Utrecht, The Netherlands

10:45 177. **LIPID-PROTEIN ASSOCIATIONS IN MEMBRANES: EVIDENCE FOR HYDROPHOBIC AND POLAR INTERACTIONS IN MITOCHONDRIAL STRUCTURE**
G. Lenaz, A. M. Sechi, G. Parenti-Castelli, J. Cabo Soler, University of Bologna, Italy

11:05 178. **INTRACELLULAR TRANSPORT OF PHOSPHOLIPIDS IN LIVER**
K. W. A. Wirtz, University of Utrecht, The Netherlands

11:25 179. **STUDIES OF THE LOCALIZATION OF CERTAIN LIPIDS IN SURFACE MEMBRANES OF TETRAHYMENA PYRIFORMIS**
Yoshinori Nozawa, Raymond Bambery, Annita Weidenbach, G. A. Thompson, Jr., University of Texas

11:45 180. **STRUCTURAL ASPECTS OF ERYTHROCYTE MEMBRANE PROTEINS**
P. Zahler and E. Wehrli, Theodor Kocher-Institute, Freierstrasse 1, Berne, Switzerland

12:05 181. **THE INTERACTIONS OF PROTEINS ISOLATED FROM ERYTHROCYTE MEMBRANES**
A. H. Maddy, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland

12:25 **PANEL DISCUSSION: INTERRELATIONSHIPS BETWEEN THE PLASMA LIPOPROTEINS AND THE MEMBRANE PROTEINS**

THURSDAY MORNING—MAY 6

9:00 A.M.—Continental Room

SESSION HH—SYMPOSIUM: ROLES OF STEROIDS IN FUNGI

Chairman—**J. W. Hendrix,** University of Kentucky

9:00 **INTRODUCTORY REMARKS**

9:10 182. **STUDIES ON THE ROLE OF STEROLS IN YEAST CELL METABOLISM**
L. W. Parks, P. R. Starr and E. Thompson, Oregon State University

9:40 183. **EFFECTS OF STEROLS ON GROWTH AND DEVELOPMENT IN PHYTOPHTHORA CACTORUM**
C. G. Elliott, University of Glasgow, Scotland

10:10 184. **STEROID NUTRITION AND METABOLISM IN PYTHIUM AND PHYTOPHTHORA**
J. W. Hendrix, University of Kentucky

10:55 185. **STEROLS IN RELATION TO NONSENSITIVITY OF PYTHIACEAE FUNGI TO POLYENE ANTIBIOTICS**
P. H. Tsao, University of California

11:25 186. **ROLE OF STEROIDS IN DIFFERENTIATION OF PHYMATOTRICHUM OMNIVORUM**
H. E. Bloss, University of Arizona

ABSTRACTS OF PAPERS

WAX ESTER METABOLISM IN FISH ROE. D. M. SAND, J. L. HEHL and HERMANN SCHLENK, The Hormel Institute, Austin, Minn. 55912.

The origin of the alcohol moieties is of particular interest in the biochemistry of wax esters. Early in studies of this lipid class the structural similarities of acids and alcohols suggested the former to be the precursors of the latter. Such relationship was proven very clearly with the gourami (*Trichogaster cosby*). This fish incorporates dietary fatty acids efficiently into triglycerides and the other common lipids of body fat, but incorporates them at even higher levels as acids and alcohols into wax esters of the roe. Incubation of gourami eggs with palmitic acid showed efficient incorporation of the chain into wax esters as acid and alcohol moiety. The extent of reduction was the same as in experiments in vivo. Dietary alcohols are incorporated by the fish as acids into body lipids, but also as alcohols and acids into roe wax esters. The use of U-^{14}C 16:0 and 18:1 alcohols labeled additionally with ^{3}H in C-1 showed that the greatest portion of these alcohols, when incorporated into roe wax esters, undergoes an oxidation-reduction cycle since most of the ^{3}H is lost. About half of the ^{3}H preserved in lipids resides in the alcohols of wax esters. The other part is in triglycerides and glycerol phospholipids and there it is nearly exclusively in the glycerol moieties. After abstraction from the alcohol group, ^{3}H is reused for reduction, most likely of triosephosphate. Such reuse for reduction is also indicated by ^{3}H appearing in C-1 of alcohols other than those fed. In contrast to the highly active interconversion of the functional groups, chain conversions are very slow. Both male and female gouramis retained one half to two thirds of ^{14}C in their lipids four months after ^{14}C-18:1 acid had been fed. More than 90% of this was still in 18:1 chains. The ratio of ^{14}C in 18:1 alcohol-acid in wax esters remained constant, but ^{14}C-18:1 diminished in roe and shifted in part to the triglycerides of the body.

2

ENZYMATIC SYNTHESIS OF LONG CHAIN ALCOHOLS AND WAX ESTERS FROM FATTY ACIDS. P. E. KOLATTUKUDY, Washington State University, Pullman, Wash. 99163.

Long chain alcohols and wax esters are widely distributed in nature. Synthesis of fatty alcohols from fatty acids (C_{12}-C_{28}) was studied with partially purified enzymes from *Euglena gracilis* and *Brassica oleracea*. With the *E. gracilis* enzyme preparation fatty acid reduction to alcohol required ATP, CoA and NADH. Although aldehyde intermediate did not accumulate, its formation could be demonstrated with the use of phenyl hydrazine as a trapping agent. The same preparation reduced fatty aldehyde to alcohol with either NADH or NADPH as the reductant. Apparent Kms for myristic acid and NADH were 2.4×10^{-4}M and 1.6×10^{-5}M, respectively, and the pH optimum was about 6.5. A partially purified enzyme from young leaves of *B. oleracea* reduced fatty acyl CoA to aldehyde with NADH as the reductant. Another protein fraction from *B. oleracea* catalyzed the reduction of fatty aldehydes to alcohols with NADPH as the preferred reductant. The aldehyde reductase showed a pH optimum at 5.6 and apparent Km for NADPH and palmitaldehyde were 5×10^{-5}M and 7×10^{-5}M, respectively. All the reductases mentioned above were severely inhibited by SH reagents such as parachloromercuric benzoate, N-ethyl maleimide and iodoacetamide. Fatty alcohols were enzymatically esterified with fatty acids by three different mechanisms in *B. oleracea*, where the source of acyl group was free fatty acids, phospholipids or acyl CoA. These different enzymatic activities have been separated, partially purified and characterized to a limited extent. Cell-free preparations of *Euglena gracilis* catalyzed wax ester synthesis from fatty acids and alcohols only in the presence of ATP and CoA showing that the acyl CoA-linked esterification is the predominant mechanism for wax ester synthesis in this organism.

3

THE BIOSYNTHESIS OF WAX ESTERS: ENZYMIC INTERCONVERSIONS OF FATTY ACIDS AND FATTY ALCOHOLS. FRED SNYDER, Oak Ridge Associated Universities, Oak Ridge, Tenn. 37830, and BOYD MALONE.

Fatty alcohols that serve as precursors of wax esters and ether-linked chains of glycerolipids are formed by reductases that utilize fatty acids as substrates. The enzymic reduction of 1-^{14}C-palmitic acid to hexadecanol and the enzymic oxidation of 1-^{14}C-hexadecanol to palmitic acid have been demonstrated in mammalian cells. NAD is required for oxidation. NADH was much less effective than NADPH as a source of hydrogen in the reductive system; however, combining both reduced nucleotides produced the highest yield of fatty alcohol. ATP, CoA and Mg^{++} are also required for the reduction of fatty acids (presumably for activation) but they are not necessary for the oxidation of fatty alcohols. Oxidation of hexadecanol was inhibited to some extent by ATP, CoA and Mg^{++} since these cofactors apparently activate endogenous fatty acids used for the biosynthesis of waxes. This competing reaction reduces the available substrate for the oxidation to fatty acids, i.e., 63% of the total ^{14}C-activity was found as waxes. Essentially no waxes were formed (<0.57% of the total ^{14}C-activity) in the absence of ATP, CoA and Mg^{++}.

4

UNUSUAL WAX ESTERS FROM PORPOISE ACOUSTIC AND NONACOUSTIC TISSUES. USHA VARANASI and D. C. MALINS, National Marine Fisheries Service, Seattle, Wash. 98101.

Wax esters from porpoises are unique with respect to previously studied terrestrial and aquatic plants and animals. Furthermore, striking differences in wax ester profiles between acoustic and nonacoustic tissues suggest an important role for certain lipid structures in sound transmission, a vital function in the biosonar capability of these animals. Wax esters of porpoises are primarily isovaleryl derivatives of long chain alcohols. High percentages of long chain acids and alcohols containing iso structures are also present. In addition to isovaleric acid, wax esters from the blubber (nonacoustic tissue) comprise large amounts of unsaturated acids, primarily 16:1 and 18:1 structures. Furthermore, small proportions of long chain saturated iso acids are also present. However, wax esters from the mandibular canal and melon (acoustic tissues) contain two to three times as much isovaleric acid, four times as much long chain saturated iso acids, and relatively small proportions of unsaturated acids. Furthermore, alcohols obtained from wax esters of acoustic and nonacoustic tissues showed similar diversities in distribution patterns with respect to iso and unsaturated structures. Studies conducted so far on the occurrence of isovaleric acid and iso structures in wax esters of porpoises will be summarized with respect to dietary influences and the projected role of leucine in biosynthesis.

5

WAX ESTER LIPASES OF MARINE ANIMALS. ELIZABETH A. BAKER, J. C. NEVENZEL, Scripps Institute of Oceanography, Los Angeles, Calif. 90024, and A. A. BENSON.

Wax esters are major lipid constituents of many marine animals, particularly midwater pelagic animals. We have therefore examined the tissues of marine invertebrates and fishes for enzymes capable of hydrolyzing these esters. Using the release of radioactive fatty acid from a synthetic substrate as an assay, we have examined the digestive glands, liver, red muscle and white muscle of larger fishes or whole bodies of smaller fishes and copepods. Commercial porcine pancreatic lipase served as a reference enzyme source. The clear intermediate layer obtained by centrifugation at 27,000 × *g* and 2 C of dilute phosphate buffer homogenates of fresh or frozen tissues served as the crude enzyme preparation and could be partially purified by dialysis or ammonium sulfate precipitation. The pH optimum for hydrolysis of wax esters was about 7.0. Because of its availability and importance in the marine food chain, most of the work was done with epipelagic anchovy, *Engraulis mordax*. The pyloric caecum was the richest source of lipase activity; crude preparations were twice as active as those from liver and three times as active as those from red muscle; white muscle had no lipase activity. Similar results were obtained with a second epipelagic species, the jack mackerel, *Trachurus symmetricus*. The fish lipases were also active in hydrolyzing triglycerides, the wax-ester-splitting activity being about twice that for triglyceride hydrolysis; by contrast, the porcine pancreatic lipase in 1 hr released about 3.5 times as much fatty acid per milligram protein from triglyceride as from wax ester in a single experiment. The wax ester lipase activity detected in the tissues of mesopelagic fishes was low. Only triglyceride lipase activity has so far been demonstrated in copepods.

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WAX ESTER METABOLISM DURING THE DEVELOPMENT OF COPEPODS. R. F. LEE, Scripps Institute of Oceanography, LaJolla, Calif. 92037, JED HIROTA, J. C. NEVENZEL, A. R. LEWIS and A. A. BENSON.

Wax esters have been shown to be important energy reserves in many of the marine calanoid copepods. Since previous studies had been done on adults we decided to investigate the lipids of the earlier stages. Copepods have a complicated life history involving egg, six naupliar stages, and finally six copepodite stages (the sixth copepodite stage is the adult). In *Calanus helgolandicus*, a common copepod in waters off western United States, we found no wax esters in the egg, six naupliar stages, or first copepodite stage (C-I). Wax ester first occurred in the late second copepodite stage (C-II) and in increasing amounts in the later copepodite stages. The C-V showed the highest amount of lipid and wax esters. The gradual build up of reserve type lipid parallels the observed increase in the oil sac in later copepodite stages. The naupliar stages do not show an oil sac. *Paraeuchaeta*, a deep water copepod in which the adult contains large amounts of wax (80% of its lipid), was found to have egg sacs containing triglyceride as the only major lipid (80% of the dry weight). Recent studies have been completed with *Euchaeta japonica*, a common copepod in inlets near Vancouver, British Columbia. All life history stages of *Euchaeta* showed the presence of wax esters. There was a gradual decrease in the amount of wax esters in the naupliar stages, and then an increase in wax esters in copepodite stages, with C-V containing the highest amount of wax ester. Of all copepod stages examined the C-V possessed the highest lipid content (per cent of dry weight), and also the greatest amount of wax ester (expressed as per cent of lipid). These observations are consistent with the natural history of copepods. The C-V is the over-wintering stage; extensive feeding takes place in all stages up to C-V. The C-V then descend in the water column and utilize the wax esters as a food reserve during a food starvation period. In the spring the adults form from the C-V, and the females rapidly use the wax ester for the formation of eggs. The adults of *Calanus plumchrus*, a North Pacific copepod, have no feeding mouth-parts so that all energy for the adult must come from stores of wax ester.

7

SYNTHESIS AND CHARACTERIZATION OF 9,10-METHYLENE-10-OCTADECENE, AN ANALOGUE OF STERCULENE. J. R. CLARK, University of Arizona, Tucson, Ariz. 85721, and H. W. KIRCHER.

Hypoglycin (3-methylenecyclopropyl alanine) produces severe hypoglycemia in a variety of mammalian systems through its inhibition of β-oxidation of fatty acids. Inhibition is believed to be the result of the formation of acyl CoA and acylcarnitine derivatives which are slowly metabolized, thus lowering the levels of free CoA and carnitine available for β-oxidation. Sterculic acid [ω-(2-*n*-octylcycloprop-1-enyl) octanoic acid] has an entirely different and perhaps unrelated biological activity. Its mechanism of action appears to be primarily centered around a disturbance of normal membrane permeability. Permeability alteration serves to rationalize the pink-white phenomenon in avian eggs, the retardation of the maturation of female rat

reproductive tissue and the postmortem observations of stillborn fetus from rats fed Sterculia foetida oil during pregnancy. It is still inconclusive however, as to whether sterculic acid exerts a direct effect on the membrane through surface interaction, by direct incorporation into the membrane structure, or indirectly by changing the quantity of saturated fatty acids involved in the membrane phospholipids. In any case, it is not known why hypoglycin and sterculic acid, having such chemically similar primary functional groups, have such drastically different mechanisms of action. Two explanations have the most credibility. The first is that sterculic acid is not catabolized to a product which will bind CoA and carnitine and not undergo further metabolism. The second is that hypoglycin or its metabolic products do not have all the structural requirements necessary for stearic acid desaturase inhibition. The second explanation is the most accessible to resolution. For this reason, 9,10-methylene-10-octadecene was synthesized via a five step reaction sequence to be tested for its ability to inhibit the desaturation of stearic acid. Synthesis of 9,10-methylene-10-octadecene began with the tosylation of purified oleiyl alcohol. The tosylate was reduced with lithium aluminum hydride to 9-octadecene. Following purification by distillation and $AgNO_3$ column chromatography, 9-octadecene was converted to the respective gemdichlorocyclopropane derivative with sodium methoxide and ethyl trichloroacetate. 1,1-Gemdichloro-2,3-dioctylcyclopropane was then reacted with *n*-butyllithium to from 1,3-dioctylallene. The allene was subjected to the Simmons-Smith reaction to form the desired methylenecyclopropane.

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THERMOGRAVIMETRIC OBSERVATIONS ON THE FORMATION OF Δ^2-OXAZOLINES FROM β-HYDROXYALKYLAMIDES.

W. E. PARKER, T. A. FOGLIA and G. MAERKER, E. Utiliz. Res. Dev. Div., ARS, USDA, Philadelphia, Pa. 19118.

The formation of Δ^2-oxazolines from long chain β-hydroxyamides has been studied by thermogravimetric analysis. The principal reactants were the *threo* and *erythro* isomers of 9-hydroxy-10-(N-acylamino)octadecane, RCH(OH)-CH(NHCOR')R, where R' represents methyl, chloromethyl, *n*-heptadecyl, phenyl, *p*-methoxyphenyl and *o*- or *p*-nitrophenyl. Other reactants were the *threo* and *erythro* isomers of 3-hydroxy-4-*p*-nitrobenzamidohexane and *erythro* 3-hydroxy-4-benzamidohexane. When R' is an alkyl group the order of reaction was 1. When R' is an aromatic group the order of reaction was either 1 or 2 depending upon the substituent in the para position. Loss of water from the β-hydroxyamides, yielding Δ^2-oxazoline, started between 195 and 220C. Although the energies of activation, E^*_a, were low, 1.1–6.4 kcal/mole, the negative entropies of activation, ΔS^*, were high, —63 to —75 cal per degree per mole, indicating that a well ordered transition state must be achieved before reaction can occur. The *threo* isomer yielded the *trans*-Δ^2-oxazoline exclusively. The *erythro* isomer yielded both the *cis* and *trans* isomers.

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EVIDENCE FOR ADDITION COMPOUNDS OF OXIDIZED TOCOPHEROL AND SOYBEAN LECITHIN.

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Evidence is presented for the formation of addition compounds of tocopherol and soybean lecithin containing linoleic and linolenic acid when adsorbed in monolayer on silica from a mixed solution in chloroform and oxidized at 80 C for 72 hr. Tocopherol was present at 7 moles % of the lecithin in the monolayer. These are analogous to linoleic acid-tocopherol addition compounds previously reported by us. The proposed addition compounds have been partially characterized by UV, IR, and chromatographic behavior. UV spectra of the mixed lecithin and tocopherol monolayers while in silica gel slurry in a solvent were obtained by the method previously reported by us for oxidized linoleic and tocopherol monolayers. The monolayer spectra show no evidence for tocopherol dimer, and very little evidence for trimer. A minor amount of quinone is indicated, but the spectrum has a maximum at 287 mμ with no other major peaks. The addition compounds are eluted from a silica chromatogram just before lecithin by chloroform-methanol mixtures and in a similar relation from Sephadex LH-20 by chloroform. IR spectra of the doubly-purified compounds show specific lecithin absorption bands (5.75 μ, 10.3 μ), together with much enhanced 3.4–3.5, 6.8, 7.25 and 9.18 μ peaks, the latter two being characteristic of d-α-tocopherol. The UV spectrum of the purified compounds showed $\lambda_{max}^{CHCl_3}$ 287 mμ, shoulder 276 mμ. Silica slurry UV spectra show a blue shift of the 287 mμ peak relative to solvent spectra. In this spectrometric system, such a shift is characteristic of aromatic rings of tocopherol, rather than diene carbonyl groups of oxidized linoleic or linolenic acid.

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RUBBER SWELL AS A FUNCTION OF FATTY ACID ESTER CHAIN LENGTH.

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A number of fatty acid esters of varying chain length were synthesized, and a study was made of the relationship between their structures and their swelling effect on synthetic rubbers. The esters evaluated were: methyl esters of caprylic, capric, lauric, myristic and palmitic acids; *n*-butyl, isobutyl, *n*-octyl and 2-ethylhexyl esters of lauric acid. Standard rubber samples designated as type H and type L were used, and tests were carried out according to Federal test methods employed in determining the swelling effect of aircraft turbine lubricants. Both rubber samples are nitrile rubbers and the swelling with the esters was higher with type L rubber than with type H. With regard to the alcohol portion of the esters, the shorter the chain the higher the swell in both types of rubber. Branching in the alcohol moiety lowered the swell. The rate of deswelling (desorption) in air of the swelled rubber samples was also studied. Deswelling and swelling rates were dependent upon the viscosity and not the structure of the swelling medium. It was observed that the rate of desorption of ester was faster for the short chain esters than for the long chain esters. Methyl myristate, methyl palmitate and the long chain laurate esters meet MIL-23699 military specifications for type H rubbers. In type L rubbers, the swelling is too high to meet specifications.

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FUTURE TRENDS IN COMMERCIAL HYDROGENATION OF TRIGLYCERIDE OILS.

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Numerous features of the partial hydrogenation of triglyceride oils appear promising for future research and process development. These areas include the following: (a) Better understanding of the mechanism of the reaction. From a commercial standpoint, it is desirable to be able to control independently the rate of hydrogenation, the selectivity ratio and the isomerization index. (b) Improved catalysts and new processes. (c) Improved reactor concepts, including both batch and flow systems. Suggestions and predictions will be made in each area.

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TERTIARY-BUTYLHYDROQUINONE TREATMENT OF CRUDE SUNFLOWERSEED OIL.

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In earlier studies on antioxidant treatments of a number of crude vegetable oils, very unexpected results were obtained with sunflowerseed oil, treated with tertiary butylhydroquinone (TBHQ), a compound having good potential as an antioxidant for polyunsaturated vegetable oils. Not only did deodorized oil produced from the TBHQ-treated, crude sunflowerseed oil appear to have a reduced oxidative stability, but response of the deodorized oil to further treatment with TBHQ was much less than expected. Also, discoloration which developed in the TBHQ-treated crude oil during storage at elevated temperatures carried through into the deodorized oil. These studies have been repeated to determine if such stability and color problems were related to the laboratory techniques used in the earlier work or if such problems can be anticipated and will preclude use of TBHQ as an antioxidant in crude sunflowerseed oil under more normal handling conditions. Confirming the earlier findings, TBHQ very effectively retarded oxidative degradation of the crude sunflowerseed oil during storage for four months at temperatures ranging from 80–145 F. Contrary to the previous findings, however, use of TBHQ in the crude oil did not result in reduction of stability of the deodorized oil, from either unstored or stored crude oil, and the deodorized oils responded very favorably to further treatment with TBHQ. Again, notable discoloration developed in the TBHQ-treated crude oil during storage, but this discoloration was completely removed by an efficient clay-diatomaceous earth bleaching system. In general, the findings from these repeat studies indicate that TBHQ can be effectively used in crude sunflowerseed oil without causing instability of color problems if proper handling and processing techniques are employed.

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CRAMBE SEED PROCESSING: DECOMPOSITION OF THIOGLUCOSIDES WITH CHEMICAL ADDITIVES.

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Crambe meal was cooked with a variety of bases and metal salts to decompose the undesirable thioglucoside, *epi*-progoitrin. The metallic salts (particularly of iron and copper) were preferred because of their greater reactivity and because they did not damage the protein like the alkalies. An unsaturated hydroxy nitrile, representing about 25% of the decomposed *epi*-progoitrin, was the major reaction product left in the cooked meal. A thionamide decomposition product, representing about 7% conversion of *epi*-progoitrin, was also observed in meals cooked with metallic salts. The thionamide was relatively unstable in moist, hot crambe meal, especially at basic pH, and may therefore be an intermediate in a complex decomposition path. Rats fed ferrous sulfate-treated crambe meal as 30% of their diet gained 70% compared to a basal control. Enlargement of thyroid, liver and kidneys was about 1.5 times the control organs. A crambe meal heated under the same conditions but without ferrous sulfate and fed at the same diet level caused 100% mortality within two weeks. The results of feeding ferrous sulfate-treated crambe meal are sufficiently encouraging to warrant further evaluation as a feed for chicks.

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COMMERCIALIZED CONTINUOUS HYDROGENATION YIELDS MAXIMUM PROFITS AND PRODUCT FLEXIBILITY.

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Abstract not available at press time.

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ORGANOLEPTIC AND OXIDATIVE STABILITY OF BLENDS OF SOYBEAN AND PEANUT OILS.

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Acceptance of an oil or blend of oils for general use as a salad and cooking oil involves many factors. Europeans have not generally accepted soybean oil products for a variety of reasons. Some of these have been associated with their country's economy. Others are related to the flavor and oxidative stability of the oil and the different odors and flavors that result from its use as both a table and cooking oil. To learn more about the problems facing increased use of soybean oil in countries that have used peanut oil in the past, blends of peanut and soybean oils (including specially processed soybean oils) were investigated. Our taste panel scored the initial flavors and odors of these oils and their blends sufficiently high on a scale of 1 to 10 to show that the oil blends were of uniform and good quality. Because tests showed that the blends containing hydrogenated soybean oil were very stable to oxidation, they would probably be used by many people as a table and cooking oil. Our taste panel recognized flavor and odor differences among the aged samples of these blends and the original oils. The panel generally scored aged peanut oil or blends having a greater percentage of peanut oil high but often not significantly above hydrogenated soybean oil or blends having a greater ratio of hydrogenated soybean oil.

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DETECTION OF AFLATOXIN USING SMALL COLUMNS OF FLORISIL. JAMES VELASCO, ARS, USDA, Beltsville, Md. 20705.

This simplified procedure for cottonseed products can be used to detect aflatoxin at the field level where thin layer chromatographic equipment is not available. Columns made from 4–6 mm glass tubing are packed with layers of ½ in. each of sand and Florisil and 1 in. of silica gel. A 2 ml fraction of ferric gel-purified extract of cottonseed or cottonseed meal containing an equivalent of 5 g sample is added to the column. Major impurities are retained on the top layer of the silica gel while the aflatoxin fraction is adsorbed on the middle layer of Florisil. When columns are viewed under short wave UV light, only the aflatoxin B fraction fluoresces. Aflatoxin can be estimated by matching the intensity of sample tubes with standard tubes. Aflatoxin B levels of about 40 μg can be detected. This would be equivalent to about 8 μg of B_1 and B_2 in a kg of seed or meal.

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USE OF GLC IN DETECTING THE PRESENCE OF ACTIVELY METABOLIZING *ASPERGILLUS PARASITICUS* IN PEANUT STOCKS. LOUISE S. LEE, ALVA F. CUCULLU, W. A. PONS, JR., So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

A technique has been developed for concentrating a major volatile metabolite from wet peanuts containing actively growing *Aspergillus parasiticus* and for detecting this metabolite by gas liquid chromatography (GLC). Shelled Spanish peanuts were adjusted to 25% moisture, inoculated with an aflatoxigenic strain of *Aspergillus parasiticus*, incubated for four days at 30 C and mixed with Segregation 1 shelled peanuts in a weight proportion of 1:70 and 1:1400 respectively. A portable vacuum system was used to sweep known quantities of room temperature filtered air over the mixtures for 1–3 min onto a trap made of 3 g of Chromosorb 101. The adsorbent was sealed in a 120 ml serum bottle and heated for 45 min at 100 C. Programmed GLC, 50–200 C at 4 C/min, was conducted on aliquots of the headspace gas over the heated adsorbent using a 6 ft × ¼ in. stainless steel column of 10% Carbowax 20M + TPA on Chromasorb WAW support. Both CO-GLC chromatography with known and mass spectrophotometric analysis of this metabolite confirmed its identity as acetone. Small amounts of acetone were detected in good quality peanuts, but there was about a 40-fold increase in the height of the acetone peak from the 1:70 mixture and a 4.5-fold increase for the 1:1400 mixture. While this system was effective in detecting the presence of small amounts of actively metabolizing moldy peanuts, similar experiments with Segregation 1, 2 and 3 shelled peanuts of 5–6% moisture content failed to show any marked difference in the acetone peak height.

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COTTONSEED STORAGE: EFFECT OF TEMPERATURE AND MOISTURE ON INCREASE OF AFLATOXIN CONTENT. M. E. WHITTEN and L. L. SMITH, ARS, USDA, Beltsville, Md. 20705.

Cottonseed contaminated with *Aspergillus flavus* and containing aflatoxins were stored at 80 F, 85 F, and at four moisture levels favorable for mold growth for periods of up to 120 days. Some lots were aerated, others nonaerated. The effect of these conditions on the increase in aflatoxins and the quality of cottonseed will be discussed.

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STUDIES ON TWO TOXIC LIPIDS PRODUCED BY *ASPERGILLUS FLAVUS*. A. A. ADEKUNLE and O. BASSIR, University of Ibadan, Ibadan, Nigeria.

Several workers have reported that, apart from the aflatoxins, the fungus *A. flavus* (Link) produced UV fluorescent oils when cultured on rice, wheat, soya and other natural foods. Two of such oils have been isolated in the pure state from a palm-sap broth containing *A. flavus* only. Spectral studies show that they are unsaturated fatty acids. One of them was found extremely toxic to chick-, duck-, swallow- and pigeon-embryos. The implications of these findings to the mechanism of action of these lipids will be discussed.

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AUTOMATIC REDUCTION OF SPECTROPHOTOMETER DATA BY SMALL COMPUTERS: APPLICATION TO THIN LAYER CHROMATOGRAPHIC ANALYSIS OF PHOSPHOLIPIDS. G. J. NELSON, Lawrence Radiation Lab., Livermore, Calif. 94550.

A computerized system has been developed to facilitate data reduction from a spectrophotometer. It is intended primarily for the analysis of phospholipid mixtures separated by two-dimensional thin layer chromatography (TLC), but has general utility as a combined spectrophotometer-computer system. The analog output from the spectrophotometer is digitized and recorded on punched paper tape. The tape is read by a desk-top computer system programmed to convert the optical density readings of the individual sample spots from the TLC plates to micrograms of phosphorus or percent of total phosphorus applied to the plate, or both. No manual calculations are necessary. The operational sequences are described and the results obtained by the computerized and manual methods are compared. The results are at least as accurate as manual methods but have improved reproducibility and smaller standard deviations. The advantages of this system are primarily in the time saved and the reduction of human errors, which occur during data transcription and manual calculations.

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A RAPID PROCEDURE FOR DETERMINATION OF RESIDUAL HEXANE IN OILSEED FLOURS. SARA P. FORE and H. P. DUPUY, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

A simple procedure is described for the determination of residual hexane in oilseed flours. A 0.04 g sample of flour is placed between two plugs of glass wool in a gas chromatograph inlet liner. The liner is placed in the inlet of the chromatograph and 80 μl of water is injected just above the glass wool which covers the sample. Volatiles which are eluted from the flour sample are then analyzed by programmed gas chromatography on a 2 ft × ¼ in. Porapak P column. Since results can be obtained within 20 min, this procedure should be very useful for following desolventization procedures.

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ANALYSIS OF VEGETABLE OILS FOR RESIDUAL SOLVENT AND VOLATILES. H. P. DUPUY and SARA P. FORE, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

A simple elution procedure was developed for the determination of residual hexane in crude and refined vegetable oils. A sample of the oil is placed between glass wool plugs in an insert of the inlet of a gas chromatograph. The insert is positioned in the injection port of the gas chromatograph, and the solvent in the oil is eluted from the oil without contaminating the column of the gas chromatograph. The concentration of residual hexane is easily determined by comparing the area of the appropriate peak of the chromatogram with a calibration chart. This technique is sufficiently sensitive that volatiles or degradation products of vegetable oils can be detected at levels below a part per million without prior enrichment.

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A HYDROGEN BROMIDE TITRATION FOR SOAPS IN FAT PRODUCTS. R. O. FEUGE, ZIGRIDA ZARINS and J. L. WHITE, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

None of the existing methods for determining soaps in oils has been found to be entirely satisfactory, and they are even less satisfactory for determining soaps in surface active products such as the monoglycerides and sucrose monoesters. A new method has been devised for the determination of alkali metal soaps by direct titration with Durbetaki reagent (hydrogen bromide dissolved in glacial acetic acid). When the titration was conducted at about room temperature in an acetic acid-benzene solution and crystal violet was used as indicator, the end point was sharp and soaps of potassium, sodium and lithium could be determined accurately in anhydrous oils, monoglycerides and sucrose esters. The presence of alcohols, glycerol and sucrose did not interfere in the direct titration. However, oxidized oils, epoxides and esters of the cyclopropene acids, which can be titrated with hydrogen bromide, did interfere. Products containing the interfering substances mentioned could be analyzed by a modified procedure. The modification consisted of dissolving the fat product in a mixture of amyl acetate and 1-butanol, washing with an aqueous solution of acetic acid, combining the washings and evaporating the water, and then titrating the residual acetates in the usual manner. The mixture of amyl acetate and 1-butanol decreased the viscosity of the fat product and prevented emulsions from forming when surface active compounds were present. The washing technique also was employed to advantage when the soap content of the fat product to be analyzed was below about 0.2%.

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A QUANTITATIVE TLC METHOD FOR THE ANALYSIS OF GERMICIDES IN PERSONAL CARE PRODUCTS. I. I. DOMSKY, Armour-Dial, Inc., Chicago, Ill. 60608, M. B. GRABER.

A previously reported qualitative TLC method for identification of germicides in personal care products has been extended and modified to enable quantitative measurements of these compounds. Using silica gel as the TLC substrate, and either benzene-ether or benzene-ether-acetic acid as the developing solvent, separations of commonly used germicides are effected. Quantitative measurements are made with a commercial TLC scanner. A near-linear relationship of peak area versus concentrations was found for each of the germicides, but for ideal quantitation, an optimum amount of each of the germicides is required.

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ANALYSIS FOR PHOSPHATE IN DETERGENTS. J. C. ABBOTT and P. H. GARRISON, JR., The Procter & Gamble Co., Cincinnati, Ohio 45217.

The AOCS Method DA 20b-57 (Analysis for Phosphate) is subject to positive interferences when used to analyze detergent products containing borax, perborate or high levels of silicate. In addition, a high bias also exists when this procedure is applied to products containing no phosphate. Because of the current interest in measuring phosphorous levels in detergent products, this paper brings the above problems to the attention of those making such measurements and suggests an alternate procedure which overcomes all of the above difficulties. The procedure is based upon a modified molybdophosphoric acid procedure in which silicate and other builders do not interfere. Comparative data obtained using the current and proposed procedures as well as data demonstrating precision and accuracy of the proposed procedure are presented.

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SYNTHESIS OF PHOSPHOGLYCERIDES. R. G. JENSEN, University of Connecticut, Storrs, Conn. 06268.

The methods employed by several investigators to synthesize phosphoglycerides and maintain stereochemical integrity will be discussed. These will include preparation of the precursors, protection of reacting groups, acylation reactions, reactions of the phosphoryl moiety, removal of protective groups, purification and proof of structure. Data will be presented from our experience with synthesis of monoacyl lecithins starting with egg lecithin and using acid anhydrides for acylation.

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ISOMERIZATION OF L-GLYCERALDEHYDE TO DIHYDROXYACETONE DURING GLYCERIDE SYNTHESIS. G. ANANDA RAO, M. F. SORRELS and R. REISER, Texas A&M University, College Station, Texas 77843.

The combined action of rat liver microsomes and glycerol kinase produces phosphatidic acid from L-glyceraldehyde in the presence of potassium palmitate, ATP, CoASH and NADH. In this process, glycerol may be produced from L-glyceraldehyde and phosphorylated to glycerol 3-phosphate, the glyceride-glycerol precursor. However, this is unlikely since, in the presence of NADH, microsomes do not reduce L-glyceraldehyde to glycerol. Another possibility is that by the action of glycerol kinase and ATP, L-glyceraldehyde 3-phosphate is produced and utilized for glyceride synthesis. This is also unlikely because the synthesis of phosphatidic acid from L-glyceraldehyde is not affected in the presence of 1-hydroxy-3-chloro-2-propanone.

phosphate, an irreversible inhibitor of triosephosphate isomerase, whereas the synthesis from DL-glyceraldehyde 3-phosphate is completely inhibited. A more likely mechanism for the phosphatidic acid synthesis is the isomerization of L-glyceraldehyde to dihydroxyacetone and subsequent phosphorylation to dihydroxyacetone phosphate which can then serve as a direct glyceride-glycerol precursor. This possibility is supported by our findings that dihydroxyacetone phosphate, instead of phosphatidic acid, is produced when potassium palmitate, CoASH and NADH are excluded from the incubation mixture. Furthermore, we have observed that microsomes isomerize L-glyceraldehyde to dihydroxyacetone. The microsomal isomerase does not act on D-glyceraldehyde. This isomerase is distinct from triosephosphate isomerase since it is active in the presence of 1-hydroxy-3-chloro-2-propanone phosphate. The dihydroxyacetone thus formed from L-glyceraldehyde can be determined spectrophotometrically by either the color produced in the presence of resorcinol or, more specifically, after its conversion to dihydroxyacetone phosphate, by measuring the oxidation to NADH in the presence of glycerol 3-phosphate dehydrogenase. Conversion of L-glyceraldehyde to either glyceride-glycerol or glycogen in intact tissues, observed by earlier investigators, may be explained by this isomerization to dihydroxyacetone and subsequent phosphorylation to dihydroxyacetone phosphate.

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BIOSYNTHESIS OF PHOSPHATIDYL GLYCEROL IN CELL-FREE EXTRACTS OF SPINACH LEAVES.

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Phosphatidyl glycerol is a major membrane component in photosynthetic tissues. The biosynthetic pathway for this phosphatide was studied in cell-free preparations of spinach leaves prepared by homogenizing leaves in a 0.5M sucrose medium containing 0.01 M phosphate buffer (pH 7.8) and 1 mM β-mercaptoethanol, followed by differential centrifugation. It was shown that L-α-glycerophosphate-^{14}C was incorporated into phosphatidyl glycerol, with intermediate formation of phosphatidyl glycerophosphate, in a system containing cytidine diphosphate diglyceride (CDP-diglyceride), Mg^{++} or Mn^{++}, Tris buffer (optimum pH, 7.3) and a 40,000 × g particulate leaf fraction, Mg^{++} or Mn^{++} and CDP-diglyceride were essential for the reaction. The incorporating activity of the 40,000 × g fraction accounted for most of the activity of the total cell-free leaf homogenate. The results show that the biosynthetic pathway for phosphatidyl glycerol in leaves is the same as that established by Kennedy for *E. coli*, i.e., CDP-diglyceride + L-α-glycerophosphate → phosphatidyl glycerophosphate + CMP → phosphatidyl glycerol + P_i.

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CHOLINE DEFICIENCY AND THE METABOLISM OF PHOSPHATIDYLETHANOLAMINE.

R. LYMAN, G. SHEEHAN and R. BABCOCK, University of California, Berkeley, Calif. 94720.

The only known means by which rats can synthesize choline is the methylation of phosphatidylethanolamine (PE). We were interested in knowing whether synthesis of particular species of PE and their metabolism was affected by a choline deficiency and also if the deficiency produced changes in the plasma phosphatidylcholine (PC) fractions. Rats were fed a soybean diet with and without choline for 7 days. They were then injected with 2-^{14}C-ethanolamine and killed at intervals up to 72 hr afterwards. Plasma PC were isolated from rats at similar intervals after injecting with Me-14C-methionine. Liver PE and plasma PC were separated into monoene, diene and hexaene fractions and distribution of the label and specific activity in the fractions determined. Total 2-^{14}C-ethanolamine incorporated into liver PE was about 28% of dose in controls and about 23% in deficient animals. Choline deficiency increased the proportion of the PE hexaene fraction and decreased that of the tetraene. Two hours after injecting ^{14}C-ethanolamine, the proportion of label in the hexaene fraction in both groups was similar (45%) but larger than in the other fractions. By 5 hr the proportion in the controls had decreased to 20%, whereas that in the deficient animals had increased to a maximum (52%), and then slowly decreased. The proportion of labeling in the tetraene fraction slowly increased until by 72 hr it contained 70% of the label in both groups. The diene fraction never contained more than 25%. Determination of the biological half-life from specific activities of each of the PE fractions indicated that the shortest was the hexaene, and the longest, the tetraene fraction, and that choline deficiency decreased turnover of all fractions. In plasma, the major PC fraction was the diene (54.7%) and choline deficiency increased it to 61.4%. This fraction also had the highest proportion of radioactivity. Choline deficiency depressed the amount and labeling of the tetraene fraction. Thus, choline deficiency decreased the turnover of hepatic PE fractions, presumably by reducing their methylation to form PC. The increased proportion of hexaene fraction in the PC suggests that the deficiency affected this fraction more than others, but the reasons for it are not known.

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GLYCOLIPID METABOLISM IN TUMORIGENIC VIRUS-TRANSFORMED CELL LINES.

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There is a profound change in the ganglioside composition of DNA virus-transformed cells compared with that in control cell lines. This alteration is characterized by a striking decrease in the concentration of higher ganglioside homologs, i.e., gangliosides with an oligosaccharide chain larger than sialyllactose. We have recently demonstrated that this change is due to a block of a specific aminosugar transferase in tumorigenic DNA virus transformed mouse cell lines. The enzyme involved catalyzes the transfer of N-acetylgalactosamine from uridine diphosphate N-acetylgalactosamine to hematoside (N-acetyl- or N-glycolylneuraminyl-galactosylglucosylceramide). These studies have now been extended in several directions. The first is an examination of ganglioside pattern and hematoside N-acetylgalactosaminyltransferase activity in "flat revertant" cell lines obtained by special techniques from DNA-transformed cell cultures. These cells have an essentially normal ganglioside pattern and the activity of the aminosugar transferase is similar to that in nontransformed control cell lines. Secondly, we have examined the ganglioside composition and transferase activity in RNA-transformed cell lines. The results obtained in these experiments will be presented at this conference.

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SILVER NITRATE THIN LAYER CHROMATOGRAPHY OF PHOSPHOLIPIDS. SEPARATION OF PIG AND SHRIMP PHOSPHATIDYLCHOLINES AND PHOSPHATIDYLETHANOLAMINES.

J. M. SHAW and N. R. BOTTINO, Texas A&M University, College Station, Texas 77843.

The fractionation of phospholipids according to degree of unsaturation by means of silver nitrate thin layer chromatography can be improved considerably by the following: using a silver nitrate concentration of 13–14% relative to silica gel; activating the 0.75 mm-thick layers at 195 C for 2½ hr; and developing the chromatograms with a solvent system consisting of chloroform-methanol-propanol-0.5% acetic acid (55:30:5:7) at 4 C for phosphatidyl ethanolamine (PE) and −10 C for phosphatidyl choline (PC). The above conditions were applied to the fractionation of PC and PE from swine liver and shrimp. The separations were reproducible as judged by the fatty acid composition of the fractions obtained. Furthermore, when the amount of each fraction was evaluated by its phosphorus content and the fatty acid compositions of the original PC and PE were recalculated, the results compared very satisfactorily with the direct determinations.

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POSITIONAL DISTRIBUTION OF THE FATTY ACIDS IN THE PHOSPHOLIPIDS OF BOVINE RETINA ROD OUTER SEGMENTS.

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The positional distribution of the fatty acids in the major phospholipids of bovine retina rod outer segments was determined. Phosphatidyl ethanolamine and phosphatidyl serine have mostly saturated acids in the 1 position and docosahexenoic acid in the 2 position. These phospholipids contain 94% and 79%, respectively, of polyunsaturated acids in the 2 position. Phosphatidyl choline contains mostly saturated acids in the 1 position, but has significant quantities of palmitic in the 2 position along with docosahexenoic acid. The levels of docosahexenoic acid in rod outer segment phospholipids are among the highest yet reported for membrane phospholipids, amounting to 23% in phosphatidyl choline, 39% in phosphatidyl ethanolamine and 45% in phosphatidyl serine.

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INTRODUCTORY REMARKS.

FREDERICK BERNHEIM, Duke University Medical Center, Durham, N.C. 27706.

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CHEMICAL REACTION MECHANISMS OF AUTOXIDATION OF POLYUNSATURATED LIPIDS.

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Lipids containing fatty acids with two or more 1,4 carbon-carbon double bonds are quite susceptible to oxidation at ambient temperatures in air. The mechanism of the oxidation process of polyunsaturated esters, including inhibition, acceleration and leveling-off stages, will be discussed. Each portion of the reaction-curve, obtained by plotting either appearance of an oxidation product or absorption of oxygen by the polyunsaturated lipid versus time, will be described, where possible, in terms of chemical reactions. Also, some of the difficulties of obtaining either chemical or physical evidence for the chemical process occurring during the inhibition stage of the reaction will be described. Some new, as well as currently accepted mechanisms will be advanced for formation of polymeric and lower molecular weight compounds resulting from hydroperoxide decomposition. The lower molecular weight compounds include: aldehydes, alcohols, hydrocarbons, olefins, acetals (cyclic and acyclic), aldehyde trimers, epoxides, esters and acids. Also, attempts will be made, where sufficient knowledge exists, to relate currently accepted autoxidation theories of polyunsaturated acids to enzymatic oxidations and peroxide decomposition. Some speculation will be included about the in vivo reactions of secondary hydroperoxide decomposition products.

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TOXICITY OF LIPID PEROXIDES.

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Assessing the toxicity of lipid peroxides is difficult for a number of reasons. It can be argued that the peroxide per se is not toxic because when it is formed endogenously, it can polymerize to form ceroid which is sequestered. The toxic symptoms may therefore be the result of removal of essential unsaturated lipids and protein from the site rather than a direct effect. Administered peroxides may also polymerize as well as react in the blood with hemoglobin to produce methemoglobin. Unpolymerized molecules can break down into carbonyl compounds and aldehydes which are known to be toxic. Malonyldialdehyde could act as a cross-linking agent. Some are reduced to the corresponding hydroxy acids. If most of the toxicity of lipid peroxides can be attributed to the breakdown products then it is not possible to correlate the damage of a tissue with its peroxide content. In fact the reverse correlation could be found. The higher the peroxide content in an organ the less the damage. A brief review of the literature on lipid peroxide toxicity will be given.

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LIPID PEROXIDATION IN MODEL MEMBRANE SYSTEMS.

A. A. BARBER, LYNN S. GRINNA, DAVID MEYER and H. M. TINBERG, University of California, Los Angeles, Calif. 90024.

Nonenzymatic lipid peroxidation has been studied in this laboratory using rat liver microsomal fractions, structural protein-lipid micelle complexes, a system of aggregated microsomal lipoprotein particles and purified lipid micelles. The kinetics of the reaction is similar in all systems except the lipid micelles. The reaction in microsomal membranes is inhibited by glutaraldehyde, which cross links proteins, and is facilitated by desoxycholate, which weakens apolar bonding. The lipid protein interactions in the model systems used are considered to be primarily apolar. The similarity in peroxida-

tion kinetics in these three lipoprotein systems reflects a similarity in their bonding. Cytochrome c-phospholipid complexes, which are stablized primarily by ionic bonds, display peroxidation kinetics more like those of purified lipid systems. Data from these experiments indicate that microsomal membranes are composed of interacting lipoprotein particles and that apolar bonding plays a significant role in the interactions. The kinetics of lipid peroxidation in vitro reflects the breakdown in these interactions with subsequent loss of membrane integrity. The absence of lipid peroxidation in vivo could, therefore, be a reflection of the stability of these interactions. Lipid peroxidation in membranes from pathological tissues is being used as an indicator of membrane integrity. Aging, Vitamin E deficiency and carcinogenesis are pathological conditions being investigated in our laboratory to test the correlation between lipid peroxidation and membrane lipoprotein stability.

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LIPID OXIDATION IN BIOLOGICAL MEMBRANES. Y. HATEFI and W. G. HANSTEIN, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037.

Hydrophobic attractions make the largest single contribution to the stability of most membranes. The strength of hydrophobic attractions is directly related to the ordered structure of the surrounding water and to the large negative entropies involved in traansfer of the apolar regions of membranes into the aqueous phase. Chaotropic agents (haloacetates, SCN^-, ClO_4^-, I^-, NO_3^-, Br^-) disorder water. Consequently hydrophobic bonds are weakened, the entropic barrier for solvation of apolar components of membranes is lowered, and membrane destabilization takes place. Destabilization of certain membranous structures in the presence of chaotropic agents renders their lipids susceptible to autoxidation. This process has been studied in the mitochondrial electron transport particles (ETP), complexes I, II, III and IV of the respiratory chain and in microsomes. Depending on the nature of the membrane and the amount of chaotrope used, lipid oxidation rates as high as 360 nmoles O_2/min/mg lipid, and lipid oxidation capacities as high as 4.8 μmoles O_2/mg lipid have been recorded. Iron-sulfur proteins and cytochromes are the natural catalysts of membrane lipid oxidation; malonaldehyde is a product. During the initial phase of lipid oxidation the molar ratio of malonaldehyde produced to O_2 consumed is greater than 0.1, but this ratio drops precipitously to a stationary level of about 0.01 and 0.02 for ETP and microsomes respectively. The rate and capacity of lipid oxidation in various membranes have been compared with their lipid composition, lipid double-bond content, labile sulfide content and cytochrome content. These results will be discussed. It has also been shown that, similar to the work of others on microsomes and chloroplasts, the functional state of a membrane influences its susceptibility to lipid oxidation. These results have suggested that a nonfunctional membrane is less stable and more susceptible to lipid oxidation. That membrane instability and susceptibility to lipid oxidation are directly related has also been tested by experiments in D_2O. Since D_2O is more structured than H_2O, membranes placed in a D_2O medium are more stable and resist lipid oxidation induced by chaotropic agents to a greater extent than those placed in H_2O. Furthermore, the ability of chaotropic agents of various potencies to induce membrane lipid oxidation has been compared with their effect on the resolution of an enzyme complex and on increasing the water-solubility of a nonelectrolyte. The excellent correlations obtained have further confirmed the thesis that (a) membrane stability is directly related to the ordered structure of the surrounding water, and (b) that the effect of chaotropes in inducing lipid oxidation in submitochondrial particles and microsomes is exerted through the aqueous phase.

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EVIDENCE FOR FREE-RADICAL DAMAGE TO MEMBRANE PHOSPHOLIPIDS BY OXIDATIVE ENZYMES. P. B. MCCAY, J. L. POYER and P. M. PFEIFER, Oklahoma Medical Research Foundation, Oklahoma City, Okla. 73104.

At least two oxidoreductases in the endoplasmic reticulum of liver and other tissues have been shown to produce extensive oxidative degradation of constitutive phospholipids in this membranous system. TPNH oxidase and gulonolactone oxidase are particularly active in this regard. The nature of the degradation has been partially elucidated as a multiple oxidative chain cleavage of the polyunsaturated fatty acids situated at the β-position of the phospholipids. The products are phospholipids containing carbonyl groups and water-soluble products such as malondialdehyde. Phosphatidyl ethanolamine and phosphatidyl choline show substantial losses of polyunsaturated fatty acids when extracted from the membranes after the reaction, and a major portion of the phosphatidyl ethanolamine fraction cannot be recovered from the extracted membranes. The phospholipids obtained are capable of undergoing polymerization to form a gum-like product which is no longer soluble in lipid solvents. The mechanism of the reaction which produces these lipid alterations appears to involve the production of free radicals since a number of structurally-unrelated free radical trapping agents completely block the attack on the phospholipids. In addition, increased levels of α-tocopherol supplementation in the diet of the experimental animals decreases the susceptibility of the membrane phospholipids to oxidative cleavage. Furthermore, it has not been determined that α-tocopherol in the microsomal membrane is metabolized to a polar product prior to the attack on the microsomal phospholipids. These various observations indicate the free radicals produced by the activity of the oxidoreductases appear to be responsible for the lipid alterations and that these occur when α-tocopherol in the membrane has been decreased to a low level probably as a result of reacting with radicals formed by the activity of the enzymes.

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THE ROLE OF LIPID PEROXIDATION IN THE INDUCTION OF HEPATIC INJURY. N. R. DI LUZIO, Tulane University Medical School, New Orleans, La. 70112.

Our previous studies have demonstrated that a variety of lipid antioxidants modify hepatic injury induced in rats by the acute or chronic administration of ethanol or carbon tetrachloride. Increased lipid peroxidation, as assayed by malonaldehyde formation, conjugated dienes or reduced lipid antioxidant concentration was demonstrated to occur at specific subcellular sites prior to the induction of the chemical-induced hepatic lesion. These events were effectively modified by antioxidant administration and were correlated with the ethanol or carbon tetrachloride-induced morphological and functional alterations. The composite data suggest that the generation of free radicals induced during the metabolism of ethanol or carbon tetrachloride alters the redox state of the cell and its subcellular components with a resulting loss of lipid antioxidant activity. The ensuing lipid peroxidation process initiates the diverse chemical and ultrastructural lesions which characterize the ethanol or carbon tetrachloride-induced lesions. In vitro studies with liver homogenates also demonstrated that decreased lipid soluble antioxidant activity preceded the peroxidation process as evaluated by the thiobarbituric acid reaction or conjugated diene analysis. Further evidence stressing the importance of ethanol metabolism to the induction of fatty liver stems from the findings that the inhibition of ethanol metabolism by pyrazole is associated with normal liver triglyceride concentrations. The prevention of ethanol-induced hepatic injury following pyrazole administration occurred in the presence of elevated blood ethanol levels. The observation that ethanol, per se, is not hepatotoxic supports the lipid peroxidation-antioxidant concept of ethanol-induced liver cell injury.

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EFFECTS OF ANDROGENS ON SERUM LIPIDS AND LIPOPROTEINS. ANTAL SOLYOM, National Institutes of Health, Bethesda, Md. 20014.

The administration of androgens to subjects with normal serum lipid levels usually diminishes the concentration of high density lipoproteins and to a lesser extent increases the concentration of low density lipoproteins. In lipemic patients the hypocholesterolemic effect of androgens has been well documented. In the dog, testosterone or methyltestosterone administration lowers the concentration of both high density and low density serum lipoproteins, and reduces serum cholesterol, phospholipid and triglyceride levels as well as apolipoprotein concentration to approximately the same extent. The mechanism whereby these effects are produced has not been fully determined. However, it has been demonstrated recently that methyltestosterone treatment resulted in an impairment of the synthesis of the apolipoprotein of high density lipoproteins, in the dog, which provides a possible explanation for the above findings. In addition, the available data on the effects of androgens on cholesterol synthesis, fatty acid mobilization and triglyceride metabolism as well as the relationship between androgenicity and hypolipemic activity will be reviewed and discussed.

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ESTROGEN EFFECT AND LIPID METABOLISM. R. H. FURMAN, Eli Lilly and Company, 307 E. McCarty St., Indianapolis, Ind. 46206.

Interest in the role of estrogen in lipid transport and metabolism stems from the following: (a) marked increases in plasma and liver lipids develop as estrogens increase during pregnancy in many vertebrate species, including man; (b) increased serum lipid levels and impaired glucose tolerance may occur in women taking oral contraceptives; (c) various laboratory animals exhibit sex differences in response to cholesterol feeding; and (d) the much higher coronary artery disease mortality of men, in comparison to premenopausal women, is generally presumed to be due to a salutary effect of endogenous estrogens on serum lipid concentrations and, therefore, atherogenesis, an effect which is presumed to subside following the menopause. Estrogen administration regularly increases the concentration of high density (alpha) lipoproteins; serum triglyceride concentrations are often, but not regularly, increased. The role of estrogen-induced changes in lipoprotein lipase activity, in the synthesis of the apoprotein moiety of high density lipoprotein, in the levels of circulating cortisol, thyroxine or growth hormone and in the glucose-insulin response will be discussed as possible explanations of the observed serum lipid alterations. Epidemiologic data will be presented suggesting that the menopause does not markedly increase death from coronary heart disease among women.

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PROGESTINS, ANABOLIC-ANDROGENS, ESTROGENS: EFFECTS ON TRIGLYCERIDES AND POSTHEPARIN LIPOLYTIC ACTIVITIES. C. J. GLUECK, D. SCHEEL, J. FISHBACK and P. STEINER, Cincinnati General Hospital, Cincinnati, Ohio 45202.

Oxandrolone (OX-7.5 mg/day-12 days) an anabolic androgen, and Norethindrone Acetate (NA-5 mg/day-12 days) were given to 43 patients with familial Types III, IV and V hyperlipoproteinemia. On OX, mean plasma triglyceride (TG) fell from 647 to 369 mg/100 ml in 19 patients ($P < .05$) and was unchanged in 7 patients. In the 19 patients whose TG fell, mean post heparin triglyceride lipase (TGL) increased from 15 to 29 mμEq FFA ^{14}C/ml hr ($P < .05$). Mean post heparin lipolytic activity (PHLA) increased from .32 to .54 μEq FFA/ml/min, and mean monoglyceride hydrolase (MGH) rose from 7 to 13 μm glycerol/ml/hr ($P < .05$ for both). On NA, mean TG fell from 590 to 344 mg/100 ml in 11 patients ($P < .05$), and was unchanged in 6 others. In the patients whose TG fell, mean TGL increased from 13 to 19.5 mμEq FFA ^{14}C/ml/hr ($P < .05$). Mean PHLA rose from .32 to .40 μEq FFA/ml/min ($P < .05$), and MGH rose from 6.2 to 7.7 μm glycerol/ml/hr (NS). The fall in TG on both OX and NA was associated with qualitative decreases in chylomicron and prebeta-lipoprotein concentration on paper lipoprotein electrophoresis. Three women (two with Type IV and one Type V) had been given estrogens (E) after surgical oophorectomy. TG levels on E were 5850, 474 and 380, falling to 1061, 220 and 190 mg/100 ml 14 days after E was stopped. PHLA on E were depressed to .130, .186 and .133 μEq FFA/ml/min (normal .25–.55), and rose to .320, .230 and .332 respectively after E was stopped. Increased postheparin lipolytic activities and increments in peripheral TG hydrolysis may in part account for the TG lowering effects of OX and NA. Elevation of TG by E appears to be mediated through depression of lipolytic enzymes.

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INHIBITION OF HEPATIC TRIGLYCERIDE BIOSYNTHESIS BY CLOFIBRATE. H. J. FALLON, L. L. ADAMS and R. G. LAMB, University of North Carolina School of Medicine, Chapel Hill, N.C. 27514.

Clofibrate (CPIB) effectively lowers serum triglyceride (TG) in man and experimental animals. The mechanism of this effect was studied in male rats fed chow or a 64% glucose diet containing 0.25% CPIB for 1–14 days. Triglyceride synthesis was measured in vivo by the rate of incorporation of ^{14}C-glycerol (i.v. or i.p.) into hepatic and serum TG during a 15 min interval. Triglyceride synthesis was reduced 40–55% in rats given CPIB for 2–14 days. Serum TG levels reached a minimum at 2 days. Serum glycerol and hepatic glycerol-3-P also were decreased by CPIB. The formation of diglyceride (DG), TB and lecithin from ^{14}C-*sn*-glycerol-3-P by rat liver homogenate preparations was reduced by 1–20 mM CPIB. The esterification of glycerol-3-P with palmitoyl CoA by rat liver microsomal preparations also was inhibited by 1–5 mM CPIB whereas DG acyltransferase and acyl CoA synthetase were not inhibited. This effect could not be attributed to a release of bound palmitoyl CoA from microsomes by CPIB and was observed in the presence or absence of added albumin. The combined in vivo and in vitro data suggest that CPIB reduces hepatic TG synthesis probably by inhibition of the acylation of glycerol-3-P. This inhibition may account in part for the fall in serum TG levels during treatment with CPIB.

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IMPROVING HIGH-ERUCIC OILSEEDS: CHEMICALLY OR GENETICALLY? W. H. TALLENT, U.S. Department of Agriculture, Peoria, Ill. 61604.

Since the desirability of erucic acid in edible products is being seriously questioned, genetically developed low-erucic varieties are expected to become a major part of the Canadian and European rapeseed crops within the next few years. In contrast, the objective of work on crambe and related oilseeds in the United States is a reliable domestic source of oil high in erucic acid for industrial purposes. Whether the oil is produced for edible or nonfood uses, however, chemical and genetic research underway in Canada, the United States, and several other countries to upgrade byproduct cruciferous meals has a common objective: removal of the glucosinolates that are characteristically present in oilseeds of the mustard family and adversely affect use of the meals as feeds. Not until convenient and rapid methods were devised for chemical analysis of appropriately small samples could the variability of glucosinolate content be explored. A Polish variety of *Brassica napus* called Bronowski has been discovered that has a relatively low glucosinolate content. Subsequent crossings with conventional rapeseed varieties reveal this desirable property to be genetically independent of erucic acid production in the seed oil. In view of these advances, a large number of *Brassica* accessions are being screened for one that is well adapted to agricultural production in the United States and like crambe has a constantly high erucic content in its seed oil. Within currently available crambe germ plasm less variability has been found than in *Brassica* species. Consequently, approaches based on current chemical research seem more promising for eliminating antinutritional factors in crambe meal.

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SESAME SEED. D. M. YERMANOS, University of California, Riverside, Calif. 92502.

Abstract not available at press time.

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THE PLANT GENETICIST'S CONTRIBUTION TOWARDS CHANGING THE LIPID AND AMINO ACID COMPOSITION OF COTTONSEED. R. J. MIRAVALLE, National Cottonseed Products Association, Inc., Memphis, Tenn. 38101.

Gossypol is well known to be responsible for the troublesome dark color of cottonseed oil. It may depress growth, cause discoloration of eggs and create metabolic disturbances when fed to nonruminant animals in excessive amounts. All but a trace of seed gossypol is contained in pigment glands present in the cottonseed kernel. Genetic research conducted by USDA scientist, S. C. McMichael, led to his discovery of a glandless seeded cotton in 1954. Glandless cottonseed are essentially free of gossypol. Using McMichael's genetic lines as gene sources, cotton breeders have developed breeding programs in the last 10 years from which three commercial glandless cotton varieties have so far been released. More glandless cotton varieties are on the way. Due to the elimination of gossypol, the color of the oil and utility of the meal from glandless cottonseed is distinctly superior to that from glanded cottonseed. The advent of glandless cottonseed, its potential value in the field of human protein nutrition, and the success in breeding improved oil and protein quality in other oilseeds, have recently caused cotton geneticists to become interested in the possibility of genetically manipulating lipid and amino acid composition in cottonseed. However, specialized genetic techniques involving interspecific gene transfer or use of wild photoperiodic Uplands may be required in making such improvements.

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THE PLANT GENETICIST'S CONTRIBUTION TOWARD CHANGING THE LIPID AND AMINO ACID COMPOSITION OF OILSEEDS: SAFFLOWER. P. F. KNOWLES, University of California, Davis, Calif. 95616.

It would appear possible to use genes with small effects to increase the oleic acid content of the seed of high-oleic varieties from about 77% to above 80%. Such genes reduce linoleic acid content of the oil of high-linoleic varieties from about 77% to 70–71%. The fatty acid composition of high-oleic varieties appears to be little affected by differences in temperature during seed development. High-linoleic types respond only slightly more. Most responsive are types with about equal amounts of oleic and linoleic acids. Where responses occur, linoleic acid levels increase with lower temperatures, and oleic acid levels with higher temperatures.

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PROBLEMS IN BREEDING ALTERED LIPID AND AMINO ACID COMPOSITION OF SUNFLOWER SEED. MURRAY L. KINMAN, ARS, USDA, College Station, Texas 77840.

There have been no serious attempts to change fatty acid composition of sunflower oil or amino acid content of the protein by breeding since there has been little demand for change. Proper processing of sunflower seed results in a stable, pleasantly flavored oil which is high in polyunsaturated linoleic acid and in protein meal or flour which is highly digestible and well-balanced with regard to amino acids. To date, there has been no need to divert the limited funds available for sunflower breeding away from the primary objective of increasing edible product per cultivated area. Should changes in oil or protein composition become desirable, there should be enough genetic variation within the domestic sunflower and its wild relatives to allow most conceivable modifications which processors or the public are willing to finance.

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BREEDING FOR IMPROVED FATTY ACID CONTENT OF SOYBEAN OIL. R. W. HOWELL and C. A. BRIM, Crops and Research Division, ARS, USDA, Beltsville, Md. 20705.

A genetic source of soybean oil with low linolenic acid is needed to start a breeding program to lower or eliminate this component of soybean oil. Most of the lines in our germplasm collection have been analyzed. The lowest level of linolenic acid found is slightly less than 4.0%. Various techniques are being used to increase selection pressure and to enhance the chances of finding a lower linolenic acid line. Our results indicate a strong maternal influence on the unsaturated acids and oil content of soybeans. A paternal effect was expressed for linolenic acid in certain crosses. The strong maternal effect complicates selection based on individual F_2 seeds. Selection is also complicated by the strong environmental effect on fatty acid composition. Linolenic acid and linoleic acid contents are highly sensitive to temperature conditions. This effect must be neutralized or otherwise taken into account whenever environmental differences may exist.

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IMPROVING SOYBEAN OIL STABILITY BY PLANT BREEDING. E. G. HAMMOND, W. R. FEHR and H. E. SNYDER, Iowa State University, Ames, Iowa 50010.

Linolenic acid is frequently cited as the "cause" of poor flavor stability in soybean oil even though linolenic acid may not be a precursor of some of the important flavor compounds in oxidized soybean oil. A project for breeding soybeans of low linolenic acid content has been started at Iowa State University. A survey of available strains performed by gas chromatography revealed relatively few strains that were appreciably lower in linolenic acid than common commercial varieties. Seed irradiated with gamma rays have been planted and found to yield a high percentage of lines low in linolenic acid in the first generation. By allowing further segregation of this material and crossing low linolenic lines, we hope to obtain soybeans with truly low linolenic acid. Lipoxygenases play an unknown role in the economy of many plants but they are troublesome to soybean processors. Lipoxygenases give many soybean products an off flavor and they may contribute to the flavor instability of soybean oil. An oxygen electrode procedure has been devised for measuring the amount of the lipoxygenase that attack triglycerides in preference to free fatty acids. A survey of a number of strains indicates some variation in lipoxygenase but no very low lipoxygenase strains. A technique has been worked out for growing young beans from soybean plant tissue culture medium to study the influence of plant hormones on the fatty acid composition of maturing beans. The results indicate little fatty synthesis from sucrose under these conditions.

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STATE AND FEDERAL REGULATORY ASPECTS OF ENVIRONMENTAL CONTROL. C. W. KLASSEN, Environmental Consultant, Springfield, Illinois.

Abstract not available at press time.

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IN PLANT CONTROL OF WASTEWATERS. J. FLOYD BYRD and J. N. MCDERMOTT, Procter and Gamble, Cincinnati, Ohio 45202.

In-plant control of wastewaters requires cooperation of every operator to achieve minimization of discharge of materials to the sewer. Operator awareness and participation is best received from first line supervisors supplemented by motivational talks by environmental specialists. Residuals in tanks, lines and equipment should be removed to the highest practical extent prior to cleaning operations. Cleaning agents should be selected which will minimize creating permanent emulsions of oil that are difficult to treat. Adequate auxilliary storage tanks should be provided so that emulsions or off quality process streams can be held for later special treatment rather than being sewered. Trap tanks for flotable oil removal from individual wastewater sources are advisable where flotable oil can be removed prior to mixing with wastewaters containing caustic or emulsifying agents. Each water recirculating system should include facilities for collecting and skimming off flotables because, even if the system is for clean water, fat will leak to it someday. Means of containment or separation of spilled material should be provided for all tanks and unloading areas. Ideally either curb type or basin collecting systems should be employed so that the drainage is held, examined and deliberately released to the proper route following the neutralization or recovery steps necessary. If the wastewaters from edible oil processing are pretreated in trap tanks, so they are reasonably free of fat in flotable form, they are entirely amenable to transport and treatment in municipal systems. A fair sewer service charge system will enable both the municipality and industry to avail themselves of the economy of joint treatment.

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OIL SEPARATION: THEORY AND PRACTICE. W. J. KATZ, Rex-Chainbelt, Inc., Milwaukee, Wis. 53202.

Abstract not available at press time.

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JOINT TREATMENT OF INDUSTRIAL AND MUNICIPAL WASTES. A. F. VONDRICK, City of Phoenix, Ariz. 85026.

Abstract not available at press time.

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STEREOSPECIFIC ANALYSIS OF TRIGLYCERIDES. HANS BROCKERHOFF, Fisheries Research Board of Canada, Halifax, N.S., Canada.

Stereospecific analysis determines how the fatty acids of triglyceries are distributed over the three different positions of the glycerol. The specific problem is the differentiation of positions 1 and 3 of *sn*-glycerol. In the presently known methods, triglycerides are first degraded to mixtures of diglycerides, either by the action of a lipase or by degradation with a Grignard reagent. The isomeric diglycerides are then resolved with the help of a stereospecific enzyme, either a diglyceride kinase or (after conversion of the diglycerides to phospholipids) a phospholipase. It is thus possible to analyze, or calculate, the fatty composition for each position on the glycerol. The key for a successful stereospecific analysis is the preparation of a representative diglyceride mixture by a truly random degradation of the triglyceride. The Grignard degradation is the most reliable method, but it is not always applicable, and it is accompanied by some isomerization of glycerides. There is need for improvement in the method. Analyses of natural fats have shown most of them to be asymmetric, i.e., the composition of fatty acids in position 1 differs markedly from that of position 3.

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SYNTHESIS OF STEREOSPECIFICALLY SUBSTITUTED MONO-, DI- AND TRIACYLGLYCEROLS. DMYTRO BUCHNEA, University of Toronto, Toronto, Canada.

The syntheses of optically active acyl-*sn*-glycerols have been attempted by many investigators involving the optical resolution of the racemic intermediates possessing basic or acidic groups, as precursors of he desired optically active acyl-*sn*-glycerols. However, none of these methods has yielded optically pure acyl-*sn*-glycerols. The syntheses of pure enantiomers of stereospecifically substituted acyl-*sn*-glycerols were achieved by Baer and Fischer from 1,2- and 2,3-isopropylidene-*sn*-glycerol. These compounds were first obtained from D- and L-mannitol by methods that transfer the asymmetry of carbon atoms 2 and 5 of the mannitols to the corresponding isopropylidene-*sn*-glycerols. Thus the 1,2- and 2,3-isopropylidene-*sn*-glycerol provide the stereochemical key-substances as starting materials for the syntheses of optically active acyl-*sn*-glycerols. Procedures have now been developed that permit the synthesis of asymmetrically substituted, optically active, saturated and unsaturated 1- and 3-monoacyl-*sn*-glycerols, unsaturated 1,2- and 2,3-diacyl-*sn*-glycerols, mixed-acid 1-saturated-2-unsaturated-diacyl-*sn*-glycerols and stereospecifically-substituted mixed-acid triacyl-*sn*-glycerols. These compounds play an important role in the synthesis of phospholipids besides being useful as substrates for enzymatic studies. (a) 1-Monoacyl-*sn*-glycerols were obtained by two different synthetic pathways: (i) from L-mannitol by a relatively simple procedure via 2,3-isopropylidene-*sn*-glycerol → 2,3-isopropylidene-1-acyl-*sn*-glycerol → 1-acyl-*sn*-glycerol; (ii) from D-mannitol by a more complicated procedure via 1,2-isopropylidene-*sn*-glycerol → 1,2-isopropylidene-3-0-benzyl-*sn*-glycerol → 3-0-benzyl-*sn*-glycerol → 1-0-triphenylmethyl-3-0-benzyl-*sn*-glycerol → 1-0-triphenylmethyl-2-acyl-3-0-benzyl-*sn*-glycerol → 1-acyl-3-0-benzyl-*sn*-glycerol → 1-acyl-*sn*-glycerol. (b) 1,2-Dioleoyl-*sn*-glycerol was prepared via 3-0-benzyl-*sn*-glycerol by the following sequence of reactions: 3-0-Benzyl-*sn*-glycerol → 1,2-dioleoyl-3-0-benzyl-*sn*-glycerol → 1,2-(bis-9,10-*cis*-dibromo)-distearoyl-3-0-benzyl-*sn*-glycerol → 1,2-(bis-9,10-*cis*-dibromo)-distearoyl-*sn*-glycerol 1,2-*dioleoyl-sn-glycerol*. The 2,3-dioleoyl-*sn*-glycerol was obtained by the same sequence of reactions, but starting with 1-0-benzyl-*sn*-glycerol. (c) 1-Stearoyl-2-oleoyl-, 1-stearoyl, 1-stearoyl-2-linoleoyl- and 1-stearoyl-2-linolenoyl-*sn*-glycerol were prepared from 1-stearoyl-*sn*-glycerol by the following sequence of reactions: 1-Stearoyl-3-0-triphenylmethyl-*sn*-glycerol → 1-stearoyl-2-oleoyl-3-0-triphenylmethyl-*sn*-glycerol, 1-stearoyl-2-linoleoyl-3-0-triphenylmethyl-*sn*-glycerol and 1-stearoyl-2-linolenoyl-3-0-triphenylmethyl-*sn*-glycerol → 1-stearoyl-2-oleoyl-*sn*-glycerol, 1-stearoyl-2-linoleoyl-*sn*-glycerol and 1-stearoyl-2-linolenoyl-*sn*-glycerol, respectively. (d) The stereospecifically substituted triacyl-*sn*-glycerols were obtained from the above listed mixed-acid 1,2-diacyl-*sn*-glycerols by introduction of a third dissimilar fatty acid substituent into position 3 of the *sn*-glycerol moiety.

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METABOLIC BASIS OF NON-RANDOM STRUCTURE OF NATURAL TRIGLYCERIDES. A. KUKSIS, W. C. BRECKENRIDGE and B. J. HOLUB, University of Toronto, Toronto, Canada.

Detailed examination of the structure of progressively smaller groups of triglycerides as obtained by chromatography has indicated that all natural glycerides possess nonrandom distributions of fatty acids. It has not been possible to decide whether the uncovered specificity in fatty acid association is a result of the fractionation or represnts as yet unrecognized biochemical compatibility among selected fatty acids and positions in the glyceride molecule. To distinguish between these possibilities, the rate and specificity of incorporation of radioactive precursors into glycerides was examined in selected rat tissues in vitro and in vivo. The distribution of the total radioactivity and the specific activity of the molecular species in the biosynthetic products was determined by combined radio-thin layer and radio-gas chromatography and stereospecific analyses. In all cases, a marked interaction was noted among fatty acids and specific positions of glycerol which gave rise to glyceride structures that failed to approximate either the 1,2,3-random, the 1,3-random 2-random, the 1,2-random 3-random, or the 1-random 2-random 3-random distributions. The experimental results could be best interpreted on the basis of a biosynthesis of specific 1,2-diglycerides by all tissues, which were converted into triglycerides by a further nonrandom acylation. An exception was provided by intestinal mucosa which during the inflow of 2-monoglycerides yielded both 1,2- and 2,3-diglycerides. There was, however, more of the 1,2-isomer isolated, which also exhibited a greater specificity in its fatty acid composition. These diglycerides and to a lesser extent their 1,3-isomers became acylated further also in a nonrandom manner, which led to an apparent increase in the overall randomness of the distribution of the radioactivity. The mucosal triglycerides, however, lose their structure upon lypolysis and uptake by other tissues.

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STEREOSPECIFIC ANALYSES OF TRIGLYCERIDES FROM SEED OILS OF *LIMNANTHES DOUGLASII* AND *MONNINA EMARGINATA*. B. E. PHILLIPS and C. R. SMITH, JR., Northern Regional Research Lab., ARS, USDA, Peoria, Ill. 61604.

Brockerhoff-type analyses were carried out on these seed oils, both of which contain appreciable amounts of uncommon fatty acids. Substituent fatty acids of *Limnanthes douglasii* include *cis*-5-eicosenoic (60%), *cis*-5-docosenoic (4%), and *cis*-13-docosenoic (erucic) (17%), as well as an unusual diene, *cis*-5,*cis*-13-docosadienoic acid (12%) and C_{18} analogs of some of these acids. Stereospecific analyses of the triglycerides revealed that monoenoic acids with $\omega 9$ unsaturation show a greater preference for position 2 than those with Δ^5 double bonds. The predominant *cis*-5-eicosenoic acid is fairly evenly distributed among the three positions of *sn*-glycerol but has the lowest affinity (22%) for position 2. In *Limnanthes* seed oil, 67% of the erucic (*cis*-13-docosenoic) acid occurs at position 2, in contrast to its preference for positions 1 or 3 in cruciferous seed oils. The reaction of pancreatic lipase with the oil was much slower than with ordinary fats and oils, presumably because of the presence of acyl groups with double bonds near the carboxyl group. This problem was overcome by using 10 times the usual amount of pancreatic lipase to generate diglycerides. As shown previously, *Monnina emarginata* (family Polygalaceae) contains (*S*)-coriolic (13L-hydroxy-*cis*-9,*trans*-11-octadecadienoic) (30%) acid along with small amounts of three other oxygenated acids; these are combined in a complex array of glyceride structures, including some with estolide linkages. Stereospecific analyses of *Monnina* seed oil reveal that in the monocoriolins, (*S*)-coriolic acid occurs mainly, though not exclusively, at position 3 of *sn*-glycerol.

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GLYCERYL ETHERS IN INSECTS: BIOSYNTHESIS OF ALKYL AND ALK-1-ENYL ETHANOLAMINE PHOSPHOGLYCERIDES. E. N. LAMBREMONT, Louisiana State University, Baton Rouge, La. 70803.

The in vivo biosynthesis of glyceryl ethers was studied using the tobacco budworm moth (*Heliothis virescens*). Groups of insects in various developmental stages were injected with 1-^{14}C-acetate, 2-^{14}C-glycerol, 1-^{14}C-hexadecanoic acid, 1-^{14}C-hexadecanol, 1-^{14}C-octadecanoic acid and 1-^{14}C-octadecanol and held for periods up to 72 hr. After total lipid extraction, the phospholipids were isolated by column chromatography, and individual phospholipid classes were obtained by preparative TLC. The lipids were then analyzed by zonal TLC, quantitative photodensitometry and liquid scintillation counting to determine the amount of ^{14}C incorporated into the alkyl, alk-1-enyl and acyl moieties of the various phospholipids. Under the proper conditions, all the substrates except glycerol were incorporated into the glyceryl ether fraction. Biosynthesis of the alk-1-enyl bonded form proceded very slowly, and long incubation times were required to obtain incorporation of isotope. The principal ether-bonded lipid synthesized was found to be alkyl and alk-1-enyl ethanolamine phosphoglyceride. Small amounts of radioactivity were recovered in the choline phosphoglyceride fraction, whereas none was found in the serine or inositol phospholipids.

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TUMOR LIPIDS: CARBON NUMBER DISTRIBUTION OF TRIGLYCERIDES AND GLYCERYL ETHER DIESTERS. RANDALL WOOD, Neuropsychiatric Research Lab., V.A. Hospital, Hines, Ill. 60141.

Lipids were extracted from 10 transplantable rat and mouse tumors and the triglycerides and glyceryl ether diesters were isolated by thin layer chromatography (TLC). The carbon number distribution of each intact neutral glyceride class was determined by high temperature gas liquid chromatography (GLC). Direct evidence was obtained for the occurrence of glyceryl ether diesters in the lipids of all 10 neoplasms. Diol glycerides, shown to occur in some mammalian lipids and with a similar TLC R_f value as triglycerides and glyceryl ether diesters, were not detected. The glycerides of both classes contained a higher percentage of high molecular weight species than most normal tissues. Glyceryl ether diesters contained a higher percentage of the higher carbon numbers than the triglycerides, suggesting independent routes of biosynthesis. The carbon number distribution of each glyceride class isolated from fractions of cell organelles enriched by centrifugation was similar. The results suggest a random distribution of all molecular weight species of each glyceride class among cell organelles, although the quantity of each lipid may differ. The GLC analysis of intact neutral plasmalogens was also achieved and their retention times compared with those of the triglycerides and glyceryl ether diesters.

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COMPARATIVE LIPID PATTERNS IN TWO FRESHWATER DOLPHINS, *INIA GEOFFRENSIS* AND *SOTALIA FLUVIATILUS*. CARTER LITCHFIELD, Rutgers University, New Brunswick, N.J. 08903, JOHN KINNEMAN, R. G. ACKMAN and C. A. EATON.

The compositions of the head and blubber fats from two freshwater dolphin species indigenous to the Amazon-Orinoco river basins have been analyzed to determine if the high isovaleric acid levels found in marine dolphin head fats are altered by the freshwater environment. Head and blubber lipids from both species were fractionated into lipid classes by TLC, and then GLC was used to characterize the intact lipids, the derived fatty acids, and the derived fatty alcohols. The lower jaw fat of *Inia geofrensis* contains 53% wax ester, 45% triglyceride and 2% diacyl glyceryl ether, while its blubber fat is >98% triglyceride. All three lipid classes in the jaw fat are rich in C_{10}, C_{12}, C_{14}, and *iso* fatty acids, but contain no isovaleric or polyunsaturated acids. The fatty alcohols in the jaw wax esters are over 90% saturated and contain 9.4% 14-methyl-*n*-pentadecanol. The head and blubber lipids of *Sotalia fluviatilus* have quite a different character, since they contain substantial quantities of isovaleric acid. The melon and jaw fats are approximately half isovaleroyl wax esters and half diisovaleroyl-triglycerides and are rich in *iso*-15:0 and *iso*-16:0 acids. The blubber fat contains mainly triglycerides with three long chain acids, but 30% monoisovaleroyl- and 5% diisovaleroyl-triglycerides are also present. The pronounced differences between *Inia* and *Sotalia* lipids indicate that dolphin head and blubber fats are more closely correlated with taxonomic classification rather than environmental and dietary characteristics. *Inia* is a member of the primitive Platanistidae group of dolphins, while *Sotalia* is classified with the marine Delphinidae which are known to contain high levels of isovaleric acid.

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THE FINE STRUCTURE OF LIPOPIGMENTS. ITARU WATANABE, Indiana University Medical Center, Indianapolis, Ind. 46202.

It is well known that nerve cells and astrocytes of the brain contain lipofuscin granules. These granules are present early in infancy and increase with aging or in various neurological disorders. In such pathological conditions, other cells found in brain (glial cells and vascular pericytes) also contain lipopigments with unusual structural patterns, differing from classical nerve cell lipofuscin. These cell-specific ultrastructural features of lipopigments are also observed in other human organs, such as peripheral nerve, muscle, liver, heart, etc. There are many pathological conditions of unknown etiology which are expressed by atrophy of parenchymal cells and accumulation of lipopigments. Neuronal ceroidlipofuscinosis or Batten's Disease is one of these disorders of the nervous system and is characterized by diffuse involvement of the nerve cells with accumulation of ceroid bodies which appear to be morphologically related to lipofuscin. Electron microscopic studies of various lipopigments from human tissue will be presented and the characteristic morphological features of these pigments will be discussed.

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ISOLATION TECHNIQUES AND LIPID COMPOSITION OF LIPOPIGMENTS. A. N. SIAKOTOS, Indiana University Medical Center, Indianapolis, Ind. 46202.

A variety of pure lipopigments have been isolated from brain, heart and liver. These lipopigments have been characterized by their physical properties, e.g., specific gravity and stability, as well as their chemical composition (enzymes, cations and lipids) into two major types of lipopigments, lipofuscin and ceroid. Lipofuscin has been isolated from normal brian, heart and liver. Ceroid has been isolated from brain specimens of patients with neuronal ceroid lipofuscinosis (Batten's disease) and human cirrhotic (alcohol) liver. The techniques employed for the isolation of these particulates involve a combination of centrifugation and precipitation or destruction of contaminating structures. The lipid fraction found in both types of pigments contains high concentrations of lipid-like polymers. The concentration of these polymeric lipids vary with organ and pigment class.

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ENZYMES ASSOCIATED WITH LIPOPIGMENTS. V. PATEL, Indiana University Medical Center, Indianapolis, Ind. 46202.

Considerable evidence now available supports the hypothesis that lipofuscin granules and many of the inclusion bodies characterizing lipidoses and other "storage diseases" are residual bodies, derived from lysosomes. This view is based on the fact that acid phosphatase and other acid hydrolases are associated with lipofuscin granules. Quantitative measurement of membrane-bound lysosomal enzymes, e.g., N-acetyl-β-glycosaminidase, β-glycosidase and soluble lysosomal enzymes, e.g., β-galactosidase, α-glucosidase, was done in isolated human brain and liver lipofuscins. Significance of such measurement and properties of some lysosomal enzymes in the lipofuscins will be discussed.

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CATION COMPOSITION IN LIPOPIGMENTS. C. CHILDRESS, Kansas School of Medicine, Lawrence, Kan. 66044, A. N. SIAKOTOS.

Pure lipopigments have been shown to contain very high concentrations (relative to dry weight) of cations. The cation composition of lipofuscins and ceroid isolated from normal and pathological human organs reveals distinct differences between the two classes of lipopigments. Lipofuscins show a preference for zinc while the ceroids contain very high concentrations of iron and calcium. The exact role of these cations in pigment formation is unknown, but these ions may be collected during lysosomal sequestration process, and stored within the matrix of the residual body (lipofuscin).

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THE CURRENT STATUS OF MELANIN AND MELANOSOMES. ANGELO CANTABONI, Indiana University Medical Center, Indianapolis, Ind. 46202.

The ultrastructure and some ultrastructural enzyme-histochemical characteristics of the murine S91, B16, HP melanomas were examined in vivo and in vitro. Apart from difference in detail (number, size, shape), the premelanosomes of the cells of these melanomas are very similar to each other and organized on the same general plan as in other normal and pathologic melanocytes in mammals; namely, they are limited by a membrane and have an inner periodic structure (alternating dark and light spaces in electron micrographs) ranging from 70 to 80 A. Premelanosomes of murine melanomas are composed of rod-like units, 300 A in diameter and these rod-like units extend along the length of the premelanosome. The rods are made up of alternating disks, distinguished by their density in the electron microscope. Electron microscopic histochemical evidence will be presented for some enzymes associated with premelanosomes. These enzymes include DOPA-oxidase, acid phosphatase and peroxidase; their role in the function and origin of premelanosomes will be discussed.

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DISORDERS OF LIPID PEROXIDATION—A VIDEO TAPED CLINICAL CONFERENCE OF NEURONAL CEROID LIPOFUSCINOSIS. W. ZEMAN, Indiana University Medical Center, Bloomington, Ind. 47401.

Abstract not available at press time.

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EFFECTS OF ETHYL *P*-CHLOROPHENOXYISOBUTYRATE (CPIB) ON BILIARY SECRETION. G. L. JORDAN, JR., E. C. HORNING and M. G. HORNING, Baylor College of Medicine, Houston, Tex. 77025.

Studies of the effect of CPIB (Atromid-S) on biliary secretion have been carried out in dogs following surgical implantation of a T-tube in the common bile duct. The drug (25 mg/kg) was administered daily for 6 to 12 months. Timed collections of bile were made on fasting animals at 10 day intervals and fasting blood samples were also obtained. Cholesterol and triglyceride analyses were carried out for both plasma and bile. In addition, phospholipids (lecithin) and bile acids were measured in the bile. The chronic administration of CPIB to dogs caused marked changes in the composition of bile. The concentration in bile and measured secretion of bile acids and lecithin increased two- to threefold. The concentration and secretion of cholesterol also increased but since the concentration of cholesterol in dog bile is rather low, the increase in milligram per hour was small compared to that observed for bile acids. No significant change in plasma cholesterol or triglyceride concentration was observed. Several studies were carried out on patients after operations for cholelithiasis.

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ORAL CONTRACEPTIVES AND LIPID METABOLISM IN THE RAT. LILLA AFTERGOOD and ROSLYN B. ALFIN-SLATER, University of California, Los Angeles, Calif. 90024.

Extensive studies on the effect of the administration to mature female rats of oral contraceptives on lipid metabolism have been undertaken in our laboratory. Dose levels, ranging from those just adequate to prevent conception to approximately 20 times this amount of two anovulatory drugs were given to animals in studies designed to elucidate effects of these compounds on various lipid parameters. In all cases these treatments resulted in marked decreases of cholesterol levels in plasma and adrenal and increases in liver. The esterified cholesterol fraction was particularly susceptible to hormonal influences; cholesterol arachidonate in plasma and the docosatetraenoate ester of cholesterol in adrenals showed the most significant decreases. In livers from dosed animals, cholesterol biosynthesis in vitro from 1-^{14}C-acetate was depressed, but in adrenals, biosynthesis was significantly enhanced. Serum phospholipids were decreased and changes in serum lipoproteins were also observed. The significance of these changes will be discussed. Although the two drugs investigated were comparable as far as their activity as contraceptive agents, they varied in their effects on lipid metabolism. Also, when the effects of each of the components of a particular contraceptive drug were compared with the effects of their mixture, it was observed that while the progesterone constituent alone was without effect on rat lipids, he estrogen constituent alone yielded enhanced results. Obviously, the progestin modified the effect of the estrogen since combination of the two components was less effective than the sum of the effects of the individual components. Long range studies are now in progress.

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CLINICAL EVALUATION OF MK-185: A NEW HYPOLIPIDEMIC DRUG. C. R. SIETORI and D. L. AZARNOFF, University of Kansas Medical Center, Kansas City, Kan. 66103.

MK-185 (2-acetamidoethyl(*p*-chlorophenyl) (*m*-trifluoromethylphenoxy) acetate) is a new hypolipidemic agent which appears to be twice as potent as clofibrate. MK-185 was tested in 11 volunteers, 6 with Type II and 5 with Type IV hyperlipoproteinemia; a simplified method will be presented to distinguish between Type II and Type IV hyperlipoproteinemias by agarose gel electrophoresis. The patients received MK-185 in two daily doses of 500 mg for six weeks, and for six more weeks received a placebo, according to a double-blind cross-over protocol. At biweekly intervals, plasma cholesterol and triglycerides levels were assayed. Lipoprotein electrophoreses were obtained at the beginning and at the end of each study period. Triglyceride levels fell significantly in all patients (35–90% in Type IV patients, 10–65% in Type II). The changes in plasma cholesterol were not significant in either group. Examination of LP-electrophoresis patterns showed an increased density of the β-band in Type II patients after therapy. In addition to the effects on plasma lipids, the drug caused striking decreases in protein bound iodine and uric acid levels in all patients. These decreases could be correlated with the blood levels of the drug assayed by gas chromatography.

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EVALUATIVE STUDIES OF THE HYPOLIPIDEMIC AGENT SaH 42–348 IN ANIMALS AND MAN. L. A. KELLY, Sandoz Pharmaceuticals, Hanover, N.J. 07936, A. R. TIMMS, J. H. TRAPOLD, R. D. GRAUPNER and J. J. CILLO, Sandoz Pharmaceuticals, Hanover, N.J. 07936.

SaH 42–348 (1-methyl-4-piperidyl bis[p-chlorophenoxy] acetate) was synthesized at Sandoz Pharmaceuticals, and has been under investigation in our laboratories for the past five years. These studies have established that SaH 42–348 is effective in lowering serum cholesterol and triglycerides in rats treated with the compound incorporated into the diet. A 20–25% increase in absolute and relative liver weight was also observed. In this respect SaH 42–348 is similar to Atromid-S (clofibrate, CPIB) although SaH 42–348 is 10 times more potent. The activity of Atromid-S can be blocked by pretreatment of the animals with propylthiouracil, but the activity of SaH 42–348 is not affected. Inhibition of acetate incorporation into cholesterol of rat liver could be demonstrated with Atromid-S, but not with 42–348. However, studies of cholesterol metabolism is an isotopic steady state indicate that neither compound causes a significant change in cholesterol coming from endogenous or exogenous sources. These data suggest that the compounds either cause an increased excretion of cholesterol or a shift of cholesterol from plasma to some other compartment. Initial clinical evaluations of this compound in patients suffering from Type II, III and IV hyperlipemia confirm the animal data and indicate that 42–348 is a safe and effective agent in controlling hyperlipemia.

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THE SOCIO-ECONOMICS OF IMPROVING NUTRITION: ROLE OF GOVERNMENT AND INDUSTRY. AARON ALTSCHUL and GRAHAM WILLIAMS, U.S. Department of Agriculture, Washington, D.C.

Abstract not available at press time.

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SOME CONSEQUENCES OF THE USE OF LINEAR PROGRAMMING TECHNIQUES FORMULATING HUMAN DIETS. H. L. WILCKE, D. T. HOPKINS and L. W. DECLUE, Ralston Purina Co., St. Louis, Mo. 63102.

Abstract not available at press time.

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THE INTERRELATIONSHIP OF CHEMICAL STRUCTURE AND ENVIRONMENT TO FUNCTIONALITY OF PROTEINS. WILDA H. MARTINEZ, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

Abstract not available at press time.

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NEW CONCEPTS IN DIFFUSED AERATION. WALTER ZENTNER, Walker Process Equipment Co., Plainfield, Ill. 60544.

With the advent of the "megalopolis" creating ever increasing pollution problems, the difficulty of locating adequate sites for treatment facilities has correspondingly increased. The old adage of buying a new section of land at the outskirts of the city limits is no longer applicable. This is not only a problem for municipalities but it is a problem for industry as well. Land costs have sky rocketed for new plant construction and the old plants were generally designed to make maximum usage of available land areas for production facilities rather than waste treatment facilities. The result of these ever-increasing problems has exerted pressure in generally two directions; the first is to re-evaluate design criteria for existing treatment processes; the second is to re-evaluate and investigate the physical plant itself. The first direction is an all encompassing area resulting in research along many different paths into increased loading rates for existing systems and new processes to more efficiently handle the waste stream. The second aspect concerning the physical plant, investigates primarily tank configuration and air diffusion design. Our continued search for better designs has led us to Deep Tank aeration applying two well known principles, that of mixing deep anaerobic digestion vessels containing high suspended solids concentrations, and that of Cross Roll aeration of standard biological reactors. The combination of these principles brought about the Deep Tank development allowing smaller plan area for the system, increased O_2 transfer, efficient economical above ground circular steel reactor vessels, and "shoe-horn" installation of multiple smaller vessels to fit unusual plot plans. These advantages are obtained using standard low pressure compressors and requiring little or no additional pumping horsepower.

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OXYGEN USE IN THE ACTIVATED SLUDGE PROCESS. J. R. MCWHIRTER, Union Carbide Corp., Tonawanda, N.Y. 14150.

Abstract not available at press time.

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CONTROL OF POLLUTANTS FROM AIR AND WATER FOR THE OILSEEDS INDUSTRY. K. W. BECKER, Blaw-Knox Chemical Plants, Inc., Pittsburgh, Pa. 15222.

Due to ever increasing pressure from ecological groups and new laws, it will be essential for the oilseeds industry to encourage many programs so that pollutants in air and water can be substantially reduced. Existing technology will permit the design of plants which meet standards presently accepted by most regulatory authorities. However, in the years to come, more rigid standards toward further minimizing what are now acceptable losses of pollutants to air and water can be expected. Furthermore, more extensive precautions must be taken for future designs to prevent sudden accidental losses of large quantities of pollutants to air and water. This means revamping many designs for future plants and considerable renovation of existing plants so that they will comply with changing acceptable standards. Additional work should be started promptly for dust separation and water purification systems so that tomorrow's standards can be met. Right now we need technological break-throughs in protein whey recovery systems and in proper methods of disposal of gossypol-aflatoxin streams.

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AIR POLLUTION—MANPOWER AND TRAINING NEEDS. H. P. KRAMER, National Air Pollution Control Administration, Research Triangle Park, N.C. 27709.

Abstract not available at press time.

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DIFFERENTIAL SCANNING CALORIMETRY OF SINGLE ACID TRIGLYCERIDES: EFFECT OF CHAIN LENGTH AND UNSATURATION. J. W. HAGEMANN and W. H. TALLENT, Northern Regional Research Lab., Peoria, Ill. 61604, K. E. KOLB.

The polymorphism of 13 single-acid triglycerides with acyl group-chain lengths ranging from 16 to 22 was studied by differential scanning calorimetry. In contrast to the single β'-form generally attributed to such triglycerides, at least two intermediate endotherms were found for most samples between the least stable (α) and most stable (β) polymorphs. For saturated triglycerides, two versions of the familiar tuning fork model meet the β'-form requirement of alternate fatty acid chains in planes perpendicular to each other. The detection of three intermediate endotherms (presumably representing β'-forms) of triolein, trilinolenin, tri-*cis*-vaccenin and trierucin may be rationalized by assuming that the segments of polymethylene chains on either side of double bonds may zigzag in different planes. Four exceptions for which no evidence was found for β'-forms are tripetroselenin, tri-*cis*-6-hexadecenoin, tripetroselaidin and trielaidin. Three of these exceptions contain Δ^6-acyl groups and have in common segments with even numbers of methylene groups between carboxyl groups and double bonds. These same three triglycerides also have a longer than usual distance from the double bond in one chain across the plane of the terminal methyl groups to the double bond in the opposed chain in the tuning fork model. Tripetroselaidin and trielaidin are exceptional in still another way. Only they and trierucin exhibited significant nonconformity with an empirical relationship between melting points and heats of fusion of β-forms. Otherwise, all points in a plot of the former physical constant vs. the latter closely fit a smooth curve, the positive slope of which gets larger as the x-axis values, i.e., melting points, increase.

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THERMAL PROPERTIES OF 2-OLEODIPALMITIN AND 2-ELAIDODIPALMITIN AND SOME OF THEIR MIXTURES AS DETERMINED BY DIFFERENTIAL SCANNING CALORIMETRY. N. V. LOVEGREN, M. S. GRAY and R. O. FEUGE, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

The polymorphism of 2-oleodipalmitin (POP) and 2-elaidodipalmitin (PEP) and some of their mixtures was investigated by differential scanning calorimetry (DSC). The heat of fusion (ΔH_f), heat of crystallization (ΔH_c) and heat of transition (ΔH_t) for 2-oleodipalmitin and 2-elaidodipalmitin were determined. Four of the five previously identified polymorphs of 2-oleodipalmitin were identified by DSC. Form IV, mp 20.8 C, was not obtained. The ΔH_f's of Forms I and III, mp's 37.2 and 29.4 C, respectively, were determined. Rapid conversion to higher melting polymorphs precluded the accurate determination of heats of transformation in some instances. All four of the previously identified polymorphs of 2-elaidodipalmitin were also identified by DSC. Forms I and II, mp's 56.5 and 56.0 C, respectively, were identified by melting points. Forms III and IV, mp's 53.5 and ca. 33–35 C, respectively, converted without melting to the next higher polymorph. Transition energies of the conversions were measured. Mixtures of 2-oleodipalmitin and 2-elaidodipalmitin in 1:3, 1:1 and 3:1 ratios were examined by DSC. The 1:3 POP/PEP mixture melted sharply; the 1:1 POP/PEP mixture melted over a broad range; and the 3:1 POP/PEP mixture melted over two ranges, the lower one being short and the higher one relatively long. Examination of three mixtures of POP and PEP by DSC gave no evidence of stable lower-melting polymorphs; the mixtures rapidly converted to the higher melting stable polymorphs. Heating and cooling curves are given for the polymorphs of POP and PEP and for their mixtures.

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CONSTITUENTS OF *ECHINACEA ANGUSTIFOLIA* ROOTS WITH INSECT JUVENILIZING ACTIVITY. MARTIN JACOBSON and R. E. REDFERN, Agricultural Research Center, USDA, Beltsville, Md. 20705.

The American coneflower, *Echinacea angustifolia*, occurs as a weed in the midwestern United States. The roots of the plant have previously been shown by this laboratory to contain a powerful insecticide and a component having antitumor activity. Removal of the former by treatment of a pentane extract of the ground roots with nitromethane left a large amount of pentane-soluble oil. The oncolytic hydrocarbon, comprising 65% of this oil, was obtained by high-vacuum distillation. The remaining viscous, yellow oil showed considerable activity in inhibiting the development of yellow mealworms, *Tenebrio molitor*, when applied topically to the pupae. The active constituents were concentrated and partially resolved by column chromatography of the neutral fraction of the oil on silica gel, eluting successively with increasing percentages of ethyl ether in hexane. The juvenilizing compounds appear to be glycerides whose structures are being investigated.

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LIPID COMPOSITION OF SUBCELLULAR PARTICLES OF PUPAE OF THE BOLL WEEVIL, *ANTHONOMUS GRANDIS* BOH. A. C. THOMPSON, R. D. HENSON, R. C. GUELDNER and P. A. HEDIN, Boll Weevil Research Lab., ARS, USDA, State College, Miss. 39762.

Lipids present in the subcellular particles of pupae stage of the boll weevil, *Anthonomus grandis* Boh., were examined to determine their concentration relationships and their general fatty acid concentration. The phospholipids were separated by column and TLC techniques. Cardiolipin makes up 73% of the phospholipids in the cell cytoplasm. The phospholipids of the nuclei contain about 50% PE. The phospholipids of the microsomes are equally distributed between PC and PE. The mitochondria contain 50% cardiolipin with 20% each of PE and PC. The major fatty acid in the phospholipids of the subcellular particles is linoleic acid (C18:2). The phospholipids of the mitochondria and nuclei contain 80% unsaturated fatty acids, respectively. The principal fatty acid in the neutral lipids of each subcellular fraction is oleic acid (C18:1). The major fatty acid in the insect is linoleic acid (C18:2). The principal neutral lipids in the cytoplasm, nuclei and microsomes are triglycerides. The mitochondria contains primarily monoglycerides (38%).

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LIPIDS OF THE AVOCADO FRUIT COAT (*PERSEA GRATISSIMA*): LIPID COMPOSITION AND THE COMPONENT TRIGLYCERIDES. C. B. SHARMA and GLENDA MARTINEZ, Marshall University, Huntington, W.Va. 25701.

The *Persea gratissima* fruit coat lipids represent 11.7% of the total fresh weight of the fruit coat. Total lipid contains 98.7% neutral lipids of which triglycerides (85.9%), carotenes and sterol esters (5%), and diglyceride (5.4%) are the major components. Free fatty acids and sterols (2.4%), and monoglycerides (0.54%) are the minor components. The phospholipids constitute only 1.3% of the total lipids. The component triglycerides of avocado fruit coat oil have been determined by thin layer argentation, pancreatic lipase hydrolysis and by the gas liquid chromatography of fatty acid methyl esters and of triglycerides. Not counting isomers, the avocado oil was found to contain 18 triglycerides having zero to four (or more) double bonds per mole of glyceride. The composition of these triglycerides in the oil is as following: no double bond (5%), one double bond (8%), two double bonds (20%), three double bonds (30%), and four double bonds (37%). About 89% of the avocado oil is composed of 11 major triglycerides: 15.2% of OL_2O; 4% of OL_2P_o; 11.8% of OOL_o; 3.4% of $P_oL_oP_o$; 14.7% of OOO; 8% of OL_oP; 3.1% of OOP_o; 14.2% of OOP; 3.6% of PL_oP; 6.7% of POP; and 4.4% of PPP. The remaining 11% is formed by 19 minor triglycerides with unsaturation varying from zero to four (or more). The positional distribution of fatty acids in the triglycerides shows the 1,3-random, 2-random distribution pattern. Unsaturated triglycerides with saturated fatty acid at 2 position represent about 5% of the total triglycerides. P, palmitic acid; P_o, palmitoleic acid; O, oleic acid; and L_o, linoleic acid).

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THE DISTRIBUTION, BIOSYNTHESIS AND RELEASE OF RENAL PROSTAGLANDINS. KEITH CROWSHAW, St. Louis University Medical School, St. Louis, Mo. 63155.

Prostaglandins (PG) E_2, $F_{2\alpha}$ and A_2 have been definitively identified in rabbit renal medulla. Recent studies indicate that incubated homogenates and slices of rabbit renal medulla contain enzymes and substrate capable of yielding PGE_2 and $PGF_{2\alpha}$. Arachidonic acid added to these homogenates produced an increased yield of biosynthesized PGs and UV spectral analysis of all the isolated carboxylic acids indicated that the

biosynthesized PGs were not appreciably metabolized by any of the known pathways to biologically inactive products. In contrast, rabbit renal cortex contains little endogenous prostaglandin-like material. The biologically active $PGE_{2\alpha}$- and $PGF_{2\alpha}$-like compounds which were isolated may have been caused by contamination of the pooled cortex with small quantities of PG-containing renal medulla. Attempts to biosynthesize PGE_2 and $PGF_{2\alpha}$ in fresh, incubated homogenates of rabbit renal cortex from endogenous precursors and from added arachidonic acid were unsuccessful. Among the tissues studied to date, rabbit kidney is remarkable in having extremely high concentrations of PGs localized within a specific area of the tissue, the medulla. We have recently obtained evidence which indicates that these prostaglandins are also present in dog renal medulla. This work involved solvent extraction of pooled renal medulla and purification of the acidic lipids by column and thin layer chromatography (TLC). The small quantities of prostaglandins were detected by a combination of smooth muscle and rat blood pressure bioassay procedures which could differentiate between PGE, PGF and PGA compounds. Final characterization of these compounds as PGE_2, $PGF_{2\alpha}$ and PGA_2 was achieved by TLC characterization on $AgNO_3$-impregnated silica gel layers. These same techniques have been used to isolate and characterize PGs present in dog renal venous blood during intraarterial infusions of vasoconstrictor drugs and during renal nerve stimulation. We observed that an increased release of PGE_2 into renal venous blood during these stimuli was coincident with increases in renal blood flow. The physiological implications of these observations will be discussed.

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PROSTAGLANDIN BIOSYNTHESIS WITH SPECIAL REFERENCE TO ADIPOSE TISSUE. E. J. CHRIST, Unilever Research Laboratory Vlaardingen/Duiven, The Netherlands.

The interference of prostaglandins and the enzyme adenyl cyclase in adipose tissue has been firmly established. The enzyme system converting suitable essential fatty acids into prostaglandins E is present in adipose tissue of rat and ram and in isolated fat cells. The regulation of lipolysis by prostaglandin E may well be a physiological phenomenon according to several criteria. In some tissues, the intracellular localization of prostaglandin synthetase was investigated in greater detail with the use of marker enzymes. The synthetase behaves as a microsomal enzyme but another localization within the cell remains possible. The effect of prostaglandins on several isolated enzymes was studied. Possible consequences of the results are discussed from the point of view that prostaglandins may act only on highly integrated systems, which are mostly under some form of biological control.

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THE EFFECT OF PROSTAGLANDIN E_1 AND LINOLEIC ACID ON EXPERIMENTAL ARTERIAL THROMBOSIS IN RATS. G. HORNSTRA, Unilever Research Laboratory, Vlaardingen, The Netherlands.

In response to certain stimuli such as exposure to collagen or adenosine diphosphate (ADP), platelets can sticks to foreign surfaces (adhesion) or to each other (aggregation). These thrombotic properties of the blood platelets are supposed to be of great importance in both the etiology of and the mortality due to atherosclerosis. Recently, the well known benificial effect of linoleic acid on the atherosclerotic process was supposed to be mediated by prostaglandin E_1 (PGE_1) which is synthesized in the body from dihomo-γ-linolenic acid, derived from linoleic acid. This hypothesis is mainly based on the fact that PGE_1 is the most potent inhibitor of platelet adhesion and aggregation known at this time. Using two techniques to study platelet adhesiveness in circulating rat blood, the following supporting evidence for this hypothesis has been obtained: PGE_1 inhibits ADP-induced platelet aggregation in circulating blood very effectively; PGE_1 inhibits experimentally induced intra arterial occlusive thrombosis in vivo; on feeding rats increasing amounts of sunflower seed oil (ca. 65% linoleic acid), the growth of intra arterial occlusive thrombosis is inhibited fat dose dependent; irrespective of whether sunflower seed oil is the only dietary fat or is mixed with hardened coconut oil (linoleic acid content <1%), a significantly positive relationship exists between the amount of linoleic acid in the diet and the degree in which the formation of occlusive arterial thrombosis is inhibited. However, the amount of dietary sunflower seed oil has no appreciable influence on the ADP-induced aggregation of circulating blood platelets. The implications of this apparent discrepancy will be discussed.

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RELATIONS BETWEEN PROSTAGLANDINS AND CARBOHYDRATE METABOLISM. B. MAY, Medizinische Hochschule, Hannover, Germany.

Among a variety of different effects some prostaglandins exert powerful actions upon lipid and carbohydrate metabolism. It could be shown that prostaglandin E_7 (PGE_7) possesses insulin-like properties in vitro and in vivo besides the well known antilipolytic activities. In addition, a hyperglycemic response to PGE_7 has been described in several species in vivo. According to our own investigations, this effect is due to a direct glycogenolytic action as well as to indirect effects mediated by the liberation of catecholamines from the adrenal glands. Moreover, prostaglandins may influence corticosteroidogenesis and modify insulin secretion in vitro and in vivo. A review of the data available from the literature is given and the results of different investigations are discussed with respect to the influence of prostaglandins upon cyclic 3',5'-AMP formation.

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PROSTAGLANDIN ANTAGONISM. J. H. SANNER, G. D. Searle and Co., Chicago, Ill. 60680.

Pharmacological antagonists of natural substances account for some of our most potent and useful drugs. They are also useful as pharmacological tools to aid in elucidating the activities of the substances they antagonize. Three types of prostaglandin antagonists have been reported in the literature, 7-oxa derivatives of prostanoic acid, polyphloretin phosphate and 1-acetyl-2-(8-chloro-10,11-dihydrodibenz [b,f] [1,4] oxazepine-10-carbonyl) hydrazine (SC-19220). Evidence for specific, competitive antagonism of prostaglandins has been presented for each of these types of compounds. Stimulatory effects of prostaglandins on isolated smooth muscle preparations are inhibited, but available information suggests that relaxant effects are not. No antagonism has been demonstrated on human tissue. None of the reported compounds are highly potent. SC-19220 has been screened for general pharmacological activity; the most potent effect was demonstrated on the central nervous system. This activity, however, does not appear to correlate with antiprostaglandin potency. Speculations on possible therapeutic uses for prostaglandin antagonists include the treatment of inflammatory conditions, especially of the eye, modulation of central nervous system activities, antifertility agents, prevention of premature labor, treatment of dysmenorrhea and treatment of diarrhea.

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THE EFFECTS OF PROSTAGLANDINS ON THE HYPODYNAMIC FROG HEART COMPARED WITH THOSE OF FATTY ACIDS, EPINEPHRINE AND ADENOSINE PHOSPHATES. A. J. VEERGROESEN and J. DE BOER, Unilever Research Laboratory, Vlaardingen, The Netherlands.

The spontaneously beating isolated frog heart (Straub preparation) has been used for the study of the effects of several prostaglandins, fatty acids, epinephrine, adenosine phosphates and Ca^{2+} on the force of contraction of hearts. Hypodynamic is due to the following: increased K^{+}, decreased Ca^{2+} or increased Mg^{2+} concentration; propranolol which blocks β-adrenergic receptors; monoiodoacetic acid which blocks glycolysis and sulfhydryl groups of coenzyme A. Positive inotropic effects have been observed with prostaglandins E_1, E_2 and 2,3 (*trans*) dehydro-E_1, but prostaglandin $F_{1\alpha}$ was found to be inactive. Adenosine triphosphate and dibutyryl c.AMP, but not c.AMP, were also effective positive inotropic agents. Furthermore the effects of the depressants mentioned above were antagonized by all fatty acids investigated, ranging from acetic acid to linoleic acid, at higher concentrations, about a hundredfold of those of the prostaglandins. The effects of the fatty acids were also characterized by a latent period of 2–5 min instead of the instantaneous onset of the prostaglandin activity. Epinephrine is not active in frog heart hypodynamic due to propranolol or monoiodoacetic acid depression. The results obtained agree with the hypothesis that the effects of prostaglandins on cardiac muscle are also the result of adenyl cyclase stimulation. This has been observed by Sobel and Robison (1969) in guinea pig heart homogenates for several prostaglandins. Since fatty acids also have positive inotropic effects the question arises whether fatty acids might have the same mode of action apart from their being used as a substrate for energy production. Finally we will discuss the possibility that prostaglandins can only stimulate adenyl cyclase indirectly, e.g., via the release of intracellular catecholamine depots.

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PROSTAGLANDIN BIOSYNTHESIS IN THE HUMAN PLACENTA. P. T. RUSSELL, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229.

The biosynthesis of prostaglandins (PG) in human placental tissue has been investigated. Acetone powder preparations of placentas were incubated in buffered solution with H^3-arachidonic acid for 0, 5, and 30 min intervals. Ethyl acetate extracts of the incubates acidified to pH 2 were separated into crude fractions on silicic acid columns using increasing concentrations of ethyl acetate in benzene. The radioactivity of peaks eluting with standard prostaglandin increased with the length of incubation time. The PG-like radioactivity was further fractionated by two thin layer chromatographic systems using Silica Gel G, with and without silver nitrate, and by columns of Amberlyst 15 ion exchange resin impregnated with silver nitrate. Only radioactivity cochromatographing with standard prostaglandins in each of the four systems was considered to be enzymatically formed prostaglandin. Results from these incubations indicate that the acetone powder preparation of the human placenta is capable of prostaglandin formation. The predominate prostaglandin-like radioactivity cochromatographed with PGA_2 standard on the argentation Silica Gel G TLC system. Less radioactivity was associated with PGE_2 standard. There was no indication of $PGF_{2\alpha}$ formation. The observed formation of the PGA_2 and PGE_2 diene prostaglandins from arachidonic acid is consistent with the known pathways of prostaglandin biosynthesis.

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CRAFT-POLYMER SHRINK-PROOF TREATMENTS OF WOOL FROM OLEIC SAFFLOWER FATTY ACIDS. M. J. DIAMOND, CARL ELLIGER, GLENN FULLER and E. N. FRANKEL, Western Regional Research Lab., Albany, Calif. 94716.

In the high oleic variety of safflower, almost 80% of the fatty acids are monounsaturated and more than 90% have 9,10 unsaturation. Application of the oxo reaction, in which carbon monoxide and hydrogen add to a double bond, produces high yields of 9- and 10-formyl groups on the fatty acids of oleic safflower. Hydrogenation of the formyl groups over Raney nickel catalyst converts them to methylol groups. Triglycerides of these acids containing primary hydroxyl functions are chemically and physically similar to castor oil. Free hydroxyaliphatic acids obtained by saponification of the triglycerides in the preparation of modified graft copolymers containing long pendant side chains were used as intermediates with highly reactive end groups. Two alternative synthetic routes were explored for the synthesis of such polymers. The resultant copolymers obtained by either route are capable of binding durably with substrates containing available hydroxyl or amino groups, such as wool, to provide a treated fabric which is shrink resistant and has a soft hand.

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N,N-DISUBSTITUTED FATTY AMIDES AS PLASTICIZERS AND PLASTICIZER STABILIZERS FOR POLYVINYL CHLORIDE RESINS. FRANK C. MAGNE, R. R. MOD and GENE SUMRELL, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

Many N,N-disubstituted amides and N-acylimines are acceptable plasticizers for vinyl chloride polymers and copolymers. Suitable amide plasticizer types are the N-acylmorpholines, N-acylpiperidines, N,N-bis(2-acyloxyethyl)-amides, N,N-dialkyl and N,N-dialkoxyalkylamides. The performance advantages and disadvantages of each type will be discussed as well as the impact of the acyl moiety and N-substituent upon the per-

formance of the amide plasticizer. Recent results show that the composite acid mixture available from a natural source, a high oleic safflower oil as well as a high *trans* tall oil derived "oleic" acid are eminently suited to the preparation of compatible amide plasticizers. The ester-amide, N-methyl-N(2-oleoyloxyethyl)-oleamide obtained by the interaction of oleic acid and N-methylethanolamine is not usable as the sole plasticizer for poly(vinyl chloride). It may be employed as an extender in blends with di-2-ethylhexylphthalate or N,N-dibutyloleamide at levels somewhat higher than 50% but at less than 75% of the overall plasticizer concentration. Asbestos tile compositions are not only plasticized but also thermally stabilized by N,N-dibutyloleamide. N,N-disubstituted epithioamides and epithioglycerides function as thermal stabilizers, comparable to the analogous epoxides in effectiveness but yielding a more easily processed composition.

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METHYL 9(10)-CARBOXYSTEARATE BY CATALYTIC AIR OXIDATION OF HYDROFORMYLATED OLEATE. A. W. Schwab, E. N. Frankel and J. C. Cowan, Northern Regional Research Lab., Peoria, Ill. 61604.

Methyl 9(10)-formylstearate from hydroformylated oleate was oxidized effectively to the corresponding carboxystearate in the presence of air and metal naphthenates. Relative activity of these metal catalysts is approximately Co > Mn, Ce, Pb > Ca, Fe, Zr, Cu. At ambient conditions, up to 95% of the starting material is converted with calcium naphthenate (0.5% as Ca) after air bubbling for 24 hr. Side reactions occur with the more active catalysts and when the temperature is raised to 100 C. Side products have the same gas liquid chromatography retention as methylstearate and hydroxystearate. Calcium naphthenate minimizes the formation of side products.

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SELECTIVE HYDROFORMYLATION OF POLYUNSATURATED FATS WITH A RHODIUM-TRIPHENYLPHOSPHINE CATALYST. E. N. Frankel and F. L. Thomas, Northern Regional Research Lab., Peoria, Ill. 61604.

Soybean, safflower and linseed oils and their methyl esters were effectively hydroformylated with a rhodium and triphenylphosphine catalyst system. Both saturated and unsaturated products containing one or more formyl groups per fatty acid were produced in good yields. The product from safflower methyl esters, hydroformylated at 100 C and 1000 psi synthesis gas (H_2 + CO), proved to be a mixture of formyloctadecanoate, formyloctadecenoate and diformyloctadecanoate. At 150 C and 1500 psi synthesis gas, the formyloctadecenoate was hydrogenated and the product formed was a mixture of mono- and di-formyloctadecanoates. The unsaturated monoformyl fraction (100 C) was identified as a mixture consisting mainly of methyl 9(10)-formyl-*cis*-12- and methyl 12(13)-formyl-*cis*-9-octadecenoates. The saturated monoformyl fraction (150 C) was a more complex isomeric mixture of methyl formyloctadecanoate. When hydroformylation of polyunsaturated fats was interrupted, *cis*-unsaturated formyl oils resulted. These unique products are being investigated as derivatives potentially useful in coatings.

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NEW POLYACETAL AND POLY(ESTER-ACETAL) COATINGS FROM HYDROFORMYLATED LINSEED OIL. T. H. Khoe, L. E. Gast, E. N. Frankel and J. C. Cowan, Northern Regional Research Lab., Peoria, Ill. 61604.

Hydroformylated linseed oils, with an average of about 1 to 5 mEq aldehyde per gram of oil, were reacted with such polyalcohols as pentaerythritol and trimethylolpropane in the presence of an acid catalyst to form viscous, high molecular weight polyacetals. Dibasic acid or anhydride modified polyacetals [poly(ester-acetals)] were prepared by reacting the ditrimethylolpropane esters of the acids with the hydroformylated oils. Films of the products were cured at room temperature and at 140 C. The films showed good hardness, as well as chemical and impact resistance. Further modification of these polyacetals and poly(ester-acetals) by reacting the residual hydroxyl groups with an excess toluenediisocyanate gave the corresponding isocyanate-terminated prepolymers. Films from these prepolymers had shorter drying times and showed greater hardness than the corresponding unmodified materials.

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FUNCTIONALITY AND END USE OF PROTEINS. R. E. Morse, Rutgers University, New Brunswick, N.J. 08903.

In the preparation of protein concentrates for human food from oilseed, animal and fish sources a great deal of emphasis has been placed upon process development and economics and resultant nutritive value. This is appropriate during early developmental stages. However, the time has now come to reevaluate such products and processes in the light of end use. Attention must be paid to objectionable odors and flavors as well as functional properties. If such products are capable only of filling the role of inert "fillers" product possibilities are greatly limited. The spectre of the, "chicken and the egg" must be faced since many of the processes developed produce such an inert, nonfunctional protein and, to solve this problem, the extraction and concentration process must be redeveloped. It will be at once obvious that processes mild enough to yield a functional protein product will constitute a microbiological dilemma. Further dimensions of this problem and field experiences will be included.

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MODIFYING PROTEINS FOR MAXIMUM UTILIZATION IN THE RUMINANT. A. D. McGilliard, Iowa State University, Ames, Iowa 50010.

Much of the early work on nitrogen metabolism in ruminants has clearly established that the rumen microflora have a considerable modifying effect on the utilization of dietary nitrogen. This modifying effect may be advantageous to the animal under certain conditions, i.e., when the diet contains mainly poor quality protein or a nonprotein nitrogenous material such as urea. On the other hand, this modifying effect may not be advantageous when high quality protein is fed. The relative importance of the routes and of the pool sizes of different nitrogenous substances varies widely with different conditions. Much of the recent research has been focused on the causes of such variations and how these variations effect the nitrogen economy of the animal as a whole. From a practical standpoint most of the studies have been directed at one of two objectives: (a) to make it possible to use, effectively, a large proportion of inexpensive nonprotein nitrogen in the diet or (b) to prevent, insofar as possible, the degradation of high quality dietary protein in the rumen. Dietary protein entering the rumen is rapidly hydrolyzed to its constituent amino acids which are then rapidly deaminated. The magnitude of this degradation is closely related to the solubility of the protein in rumen fluid. For most common feedstuffs and protein supplements as much as 80% may be degraded and fermented to ammonia in the rumen. Although this ammonia may be used for microbial amino acid and protein synthesis, it also represents a possible loss to the animal because some of it is absorbed from the rumen, converted to urea and excreted in the urine. Moreover, examination of the amino acid composition, the availability of the amino acids and the biological values of the microbial protein suggest that high-quality dietary proteins are downgraded and that low-quality point limiting the degradation of dietary protein in the rumen would be advantageous under most conditions. It should be kept in mind, however, that this probably would lead to a reduced microbial population and the possibility of secondary effects on the fermentation of carbohydrate and volatile fatty acid production. Protein degradation can be reduced by feeding natural proteins which are relatively insoluble in rumen fluid, but these also appear to be poorly digested in the small intestine. Their use would not be expected to offer any advantage. Dietary proteins or amino acids, or both, in solution or suspension can be directed past the rumen by encouraging closure of the reticular groove in intact animals or can be infused postruminally in surgically modified animals, but these procedures are impractical to use other than for research. The most promising approach seems to be chemical modification of good quality proteins or coating of proteins and individual amino acids to render them resistant to microbial attack, without greatly reducing their nutritive value in the small intestine. Several experiments have shown that heat treatment (if not too severe) of proteins (casein, peanut meal, soybean meal, fishmeal) will decrease their solubility and rumen degradation and will result in improved nitrogen utilization. Formaldehyde treatment of proteins markedly reduces their solubility and susceptibility to microbial attack. Formaldehyde-treated casein seems to be well utilized by sheep; the resultant improvement in wool growth suggests an improvement in utilization of the sulfur-containing amino acids. The complexing of proteins with tannins also reduces their solubility and susceptibility to microbial attack with a resultant increase in utilization. There is some evidence, however, that certain tannins interfere with the cellulolytic activity of the rumen organisms.

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PROTEINS FROM HYDROCARBONS: ECONOMIC POTENTIAL. C. F. Feldman, Gulf Oil Corp., Houston, Tex. 77002.

Abstract not available at press time.

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RAT ADRENAL CHOLESTEROL ESTERS. B. L. Walker and J. A. Carney, University of Guelph, Ontario, Canada.

The cholesterol esters from rat adrenals, which constitute a substantial portion of the total lipids of this tissue, contain high concentrations of the polyunsaturated acids derived from linoleic acid. In addition to the more commonly occurring arachidonic acid, adrenic acid (22:4ω6) is a major constituent of this lipid fraction, a situation not observed in most other species. A dietary deficiency of linoleic acid results in the accumulation of substantial amounts of 22:3ω9 in addition to the eicosatrienoic acid (20:3ω9) found in most tissues from deficient animals. Inclusion of erucic acid (22:1ω9) in the diet results in the incorporation of large quantities of this acid in rat adrenal cholesterol esters. The incorporation of 20:3ω9, 22:3ω9 and 22:1ω9 occurs at the expense of the 20:4ω6 and 22:4ω6 but there is a net increase in the cholesterol ester content of the adrenals under these circumstances indicating an additional incorporation of these esters as well as a partial substitution of the ω6-polyunsaturated esters. The resting levels of plasma and urinary corticosteroids are lower in EFA deficient than in normal rats, and these animals exhibit a lower ability to produce corticosteroids in response to stress situations resulting from cold exposure, ACTH injection, surgical insult or starvation. A role has been postulated for the cholesterol esters of 20:4ω6 and 22:4ω6 in which they act as readily available sources of cholesterol for steroidogenesis, and this hypothesis has been tested by measuring the changes in adrenal cholesterol ester fatty acid composition under conditions of stress. Although significant decreases in cholesterol arachidonate concentrations have been observed, esters containing other fatty acids (18:1ω9, 18:2ω6, etc.) also decrease significantly under these circumstances. In some instances the 22:4ω6 content increased, in others it decreased. Whether the impaired corticosteroid production in EFA deficient rats can be attributed solely to the low levels of cholesterol arachidonate in the adrenals of these animals is debatable.

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REGULATION OF OVARIAN CHOLESTEROL ESTERS BY GONADOTROPINS. H. R. Behrman, Harvard Medical School, Boston, Mass. 02115.

Cholesterol esters are present to a proportionately greater extent in intracellular lipid droplets of rat ovarian tissue and appear to be related to steroidogenesis in this tissue. Separate enzymes which catalyze cholesterol ester synthesis (synthetase) and hydrolysis (esterase) have been assayed in ovarian tissue. Nonfunctional immature ovarian tissue contained little synthetase and esterase activity and cholesterol ester levels were low. However, injections of gonadotropins to induce luteinization produced a 50-fold increase in total synthetase activity, a 3.4-fold increase in total esterase activity and a 270-fold increase in total tissue cholesterol esters. Removal of the pituitary from luteinized animals caused a precipitous decline in enzyme activities and cholesterol esters. Prolactin, but not LH treatment, prevented the effects of hypophysectomy. LH injected 1 hr before sacrifice increased cholesterol esterase activity in animals with an intact pituitary during the time when acute stimulation of steroidogenesis by LH has been shown. Inhibition of steroidogenesis with aminoglutethimide phosphate did not prevent LH induced depletion of ovarian cholesterol esters, which indicates that activation of cholesterol esterase is a direct site of LH action and not a consequence of increased steroidogenesis. Further evidence for this con-

clusion was obtained by injecting animals with LH antiserum. A time dependent increase in ovarian cholesterol esters was observed (1.3-fold after 8 hr and a 2.2-fold increase after 24 hr) and ovarian progesterone secretion was reduced 75% from the control level 24 hr after treatment with antiserum. An integrated action between both LH and prolactin in the maintenance of ovarian steroidogenesis and cholesterol ester turnover emerges with prolactin maintaining synthetase and esterase activity and LH activating the hydrolytic enzyme, thereby releasing unesterified cholesterol which may then be converted to steroids.

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COMPOSITION AND METABOLISM OF MILK CHOLESTEROL ESTERS. T. W. KEENAN, Purdue University, Lafayette, Ind. 47907, and STUART PATTON.

The sterol ester fraction of milk is composed largely, if not exclusively, of cholesterol esters. Although the total amount of cholesterol ester in milk lipids is small, 0.025% to 0.04% of the total lipid, they display a high level of metabolic activity. Cholesterol esters in the milks of several species exhibit elevated levels of a unique group of fatty acids in comparison with other milk ester lipid classes as well as with mammary tissue and blood cholesterol esters. This group includes monounsaturated acids and acids with odd numbers of carbons. Evidence from experiments where mass doses of odd carbon acids were infused directly into the mammary gland indicated that the group of unique acids is preferentially incorporated into and retained in the cholesterol ester fraction secreted with milk. The monounsaturated homolog of the infused acid was also found in cholesterol esters but not in other milk lipid classes. Since the acyl moieties of cholesterol esters were not desaturated in the form of the intact ester, the observations suggest a tightly coupled desaturase-acyl transferase specific for cholesterol. Mammary tissue has the enzymatic capability to both acylate cholesterol and hydrolyze cholesterol esters. The acyl moieties of cholesterol esters in mammary tissue turn over rapidly and these esters accumulate in milk fat globules in a manner paralleling triglyceride accumulation. The cholesterol esters which are secreted with milk arise from more than one metabolic pool. The available evidence indicates that at least a portion of the milk cholesterol ester fraction arises from the plasma membrane of the mammary secretory cell. Possible roles for cholesterol esters in milk lipid synthesis and secretion will be discussed.

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FATTY ACID SPECIFICITY OF RAT LIVER CHOLESTEROL ESTER HYDROLASE. D. S. SGOUTAS, Emory University School of Medicine, Atlanta, Ga. 30322.

The fatty acid specificity of rat liver cholesterol ester hydrolase (EC 3.1.1.13) has been tested using individual labeled sterol esters and mixtures of esters. Among the cholesterol esters of the 16 positional isomers of *cis*-octadecenoic acid the enzyme exhibited a distinct preference for the 9-octadecenoate. With the ethylenic bond moving to either end of the carbon chain the activity gradually decreased. Comparison of the rate of hydrolysis of cholesteryl *cis*-9,10-methylene octadecenoate with cholesteryl *cis* and *trans* 9,10-octadecenoates indicated that the presence of π electrons although perceived by the enzyme was not as important as the configuration of the acyl moiety. Experiments with cholesterol esters of unsaturated fatty acids differing in the proximal and/or terminal position of the double bond showed that a proximal portion of 9-carbon atoms is a prerequisite for an optimal hydrolysis of the corresponding cholesterol ester. In addition, the enzymatic activity was shown to depend upon the chain length of the fatty acid constituent. When a series of saturated fatty acid cholesterol esters was studied, the activity increased from hexanoic to decanoic and then decreased gradually through the eicosanoic acid cholesterol ester. These observations are discussed in terms of close matching of specially shaped acyl chains to a specially shaped complementary surface in the enzymatic active site. Furthermore, a series of cholesterol esters with odd chain fatty acids were tested and their hydrolysis rates when compared to those of even chain fatty acid cholesterol esters showed alternation, a phenomenon commonly displayed by the physical properties of long chain compounds.

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METABOLISM OF CHOLESTERYL ARACHIDONATE IN THE RAT. B. J. HOLUB and A. KUKSIS, University of Toronto, Toronto, Canada.

Cholesteryl arachidonate constitutes about 50% of the total steryl esters of normal rat plasma. In order to study its metabolism, 300 g male rats were injected with radioactive arachidonic acid, cholesterol, lysolecithin and lecithin. The incorporation of label was studied in the cholesterol and glycerol esters of plasma, liver and selected other tissues. Studies with 1-^{14}C-arachidonic acid showed that plasma cholesteryl arachidonate became effectively labeled only after the start of secretion of radioactive lecithins from the liver. Although the specific activity of the cholesterol arachidonate in the liver was initially higher than that in the plasma, this could not account for the labeling of the bulk of plasma cholesterol arachidonate at later times. The increase in the radioactivity of plasma cholesterol arachidonate was concomitant with the rise in the radioactivity of plasma lecithins secreted by the liver. This suggested that the plasma cholesterol arachidonate was formed by lecithin-cholesterol acyl transferase, which was demonstrated in vitro by incubation of plasma with lecithin labeled in the 2 position with 1-^{14}C-arachidonic acid. These observations agreed with the demonstrated higher initial radioactivity of the cholesterol esters of polyunsaturated acids in plasma following injection of 4-^{14}C-cholesterol. In other experiments, 4-^{14}C-cholesterol was found to be preferentially esterified to arachidonic acid in vitro. Furthermore, plasma lysolecithin labeled with 1-^{14}C-palmitic acid in the 1 position was shown to be extensively reesterified to arachidonoyl lecithin in the rat liver. These observations are consistent with the operation of a plasma-liver lipid cycle mediated by arachidonoyl lecithin-cholesterol and lysolecithin-arachidonic acid acyl transferases. Using common assumptions, it was calculated that 1.1 μmoles/hr of lysolecithin could be generated by the formation of plasma cholesterol arachidonate, which along with the plasma lysolecithins from other sources, could provide a maximum of 0.7 μmoles/hr of arachidonoyl lecithin in the liver via direct acylation. The turnover of arachidonoyl lecithin secreted by the liver was estimated to be 2.7 μmoles/hr. It is concluded that the formation of cholesteryl arachidonate constitutes an important fate of plasma arachidonoyl lecithins, and that the plasma lysolecithins generated by this and other reactions account for a substantial proportion of the total lysolecithin used as substrate in the formation of arachidonoyl lecithins in the rat liver. It is possible that plasma cholesterol arachidonate supplies at least part of the arachidonic acid for this reaction.

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EFFECTS OF DIET ON ESTERIFICATION AND OXIDATION OF 26-^{14}C-CHOLESTEROL IN RABBITS. K. K. CARROLL and DJAJA SURIA, University of Western Ontario, London 72, Ontario, Canada.

The distribution and metabolism of intravenously injected 26-^{14}C-cholesterol was studied in rabbits on semisynthetic and commercial diets containing no fat, 15% butter or 15% corn oil. The labeled sterol was taken up rapidly by the liver in all cases, reaching a peak of 50–60% of the injected dose after 30–60 min and then declining to about 40% over the next 10 hr. The specific activity of esterified cholesterol in liver increased more slowly. On low fat diets and butter-containing diets, it approached the specific activity of the free cholesterol after 6–8 hr, but on diets containing corn oil, the specific activity of liver esterified cholesterol was still much lower than that of the free cholesterol 10 hr after injection. Excretion of respiratory $^{14}CO_2$ reached a peak between 1 and 2 hr after injection and declined thereafter. The rates paralleled the level of radioactivity in liver free cholesterol more closely than that of esterified cholesterol, suggesting that free rather than ester cholesterol is the main substrate for oxidation. The amount of label excreted as CO_2 was at least five times greater on commercial diets than on semisynthetic diets with the same fat content. Dietary fat increased the rate of excretion of $^{14}CO_2$, particularly on the commercial diet and with this diet corn oil was more effective than butter.

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INSULIN AND DEXAMETHASONE EFFECTS ON RAT LIVER CELLS IN CULTURE. L. E. GERSCHENSON, University of California, Los Angeles, Calif. 90052.

A cell line derived from adult rat liver has been established. Biochemical and morphological studies show the cells to be similar to hepatocytes. A cell strain has been developed that grows in serumless medium; insulin is a growth factor at physiological concentrations while dexamethasone is a growth inhibitor. The addition of dexamethasone to the culture medium induced a three- to sixfold increase in the specific activity of tyroxine α-ketoglutarate transaminase. Insulin is an inducer of pyruvate kinase. The effect of RNA and protein synthesis inhibitors on these effects has been studied. The hormonal effects are greatly diminished or absent in contact-inhibited but still viable rat liver cells.

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LIPID ACCUMULATION IN MAMMALIAN CELLS. C. G. MACKENZIE, JULIA B. MACKENZIE and O. K. REISS, University of Colorado School of Medicine, Denver, Colo. 80220.

The synthesis of fatty acids and cholesterol by cells is repressed by the presence of 10% serum in the growth medium as shown by labelling experiments with tritiated water. In the absence of de novo fatty acid synthesis, the lipid content of mammalian cells ranges from approximately 0.16 to 0.50 μg cell lipid per μg cell protein. A cell's position in this spectrum is independent of tis tissue or species of origin. Analysis has shown that these differences in cell lipid are due primarily to triglyceride accumulation with differences in cholesterol and polar lipid being of secondary importance. All of the accumulated triglyceride appears in particles ca. 1 μ in diameter surrounded by a limiting membrane. Consequently, mammalian cells are also distributed in a morphological spectrum based on their display of lipid rich particles or liposomes. As shown by excellent growth rates, a high lipid content along with a large number of lipid particles is not a sign of cell damage or pathology. Rabbit serum, and some samples of human serum, cause a large increase in the triglyceride content of many cell lines, vis a vis horse serum. Fractionation of rabbit serum by precipitation with ammonium sulfate or by ultracentrifugation indicates that three quarters of its lipogenic activity in bioassay experiments is associated with albumin. This conclusion is substantiated by the high lipogenic potency of albumin isolated from proteins of $d > 1.21$ in a purity of 98.5%. Of the albumin's bound lipids, only the free fatty acids, as measured by DEAE column chromatography, are present in an amount sufficient to account for the observed increases in cell triglyceride. The remainder of the lipogenic activity of rabbit serum is associated with the lipoproteins. Comparison of the serum albumin-bound fatty acids with the fatty acids of the cell's complex lipids show that the latter contain lower concentrations of C16:0 and higher concentrations of C18:0 and C20:4 than does the albumin. Also, within the cell, C16:0 is higher in the triglyceride whereas C18:0 and C20:4 are high in the polar lipid fraction. Concentrations of C18:1 and C18:2 are similar in both fractions, but the small amount of C18:3 present is confined almost entirely to triglycerides. When the albumin of whole rabbit serum is labeled with tracer 1-^{14}C-palmitic acid to give its bound palmitic acid a specific activity of 1000 dpm/μg, the palmitic and stearic acids isolated from the cell triglycerides contain 810 and 58 dpm/μg, respectively, and the palmitic and stearic acids isolated from the polar lipids contain 370 and 35 dpm/μg, respectively. Significant counts are not detected in the oleic or polyenoic fatty acids. When the oleic acid of native albumin is similarly labelled to give 1000 dpm/μg, the radioactivity in the cell's triglycerides and polar lipids is confined to oleic acid. The oleic acid isolated from these fractions contains 840 and 550 dpm/μg, respectively. The isotopic experiments indicated that approximately 80% of the fatty acids in the accumulated triglycerides are derived from the albumin-bound fatty acids of the serum. They also indicate that a very considerable number of the fatty acid molecules present in the cell's polar lipids are derived from some other source, i.e., presumably lipoproteins of the serum.

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STUDIES ON THE BIOSTABILITY OF TISSUE CULTURE CELL LIPIDS. R. P. GEYER, Harvard School of Public Health, Cambridge, Mass. 02138.

Rapidly multiplying cells in culture in vitro possess very active lipid metabolism in order to meet the demands im-

posed by the requirements for lipids in making new membranes and related cellular components. Constituents of these lipids can be derived in part from exogenous precursors and from endogenous sources. Because they multiply rapidly and can be adapted to grow in the absence of serum, Strain L fibroblasts were chosen for this work. The production of and subsequent fate of cellular lipids were studied by various methods. A number of experimental variables, including medium composition, cell density and incubation temperature, were investigated for possible influences on either the biosynthesis or biodegradation of the phospholipids, triglycerides and cholesterol. Some of the phospholipid in these cells is extremely stable from a biological standpoint and appears not to undergo significant turnover. On the other hand, triglycerides, whether present in normal or elevated amounts, have a much lower biological stability. Presently our data suggest that HTC cells have the capacity for significant conversion of glucose carbons into lipids in a growth medium containing 10% serum. Also, the cells apparently can respond to nutritional pertubations which affect their de novo synthesis of lipids. The latter is contrary to prevailing views which suggest a loss of metabolic regulation in lipid metabolism of transplanted hepatomas.

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CELLULAR CONTROL MECHANISMS FOR LIPID TRANSPORT AND METABOLISM. J. M. Bailey, George Washington Medical School, Washington, D.C.

Abstract not available at press time.

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CONVERSION OF CARBOHYDRATE INTO LIPIDS IN MALIGNANT AND NONMALIGNANT CULTURED LIVER CELLS. J. A. Watson, University of California Medical Center, San Francisco, Calif. 94101.

The conversion of glucose and fructose into lactic acid and lipids was studied in Cultured Morris Minimal Deviation Hepatoma 7288tc (HTC cells) and epithelial, contact inhibited, liver cells cloned from neonatal Buffalo rats (HC cells). It was found that HTC cells utilize approximately 70–80% of the glucose consumed for lactic acid production and glucose was readily incorporated into the total cellular lipid fraction. The percent conversion of uniformally labelled glucose into lipids was 25–33% in a 10% serum medium. Tritiated water gave a value of approximately 14%. HTC cells can consume fructose, but do not multiply. Also, no utilization or growth is apparent when glycerol is presented to the cells. When HTC cells are cultivated in growth medium with no serum they do not proliferate, but the rates of incorporation of glucose-^{14}c(U) into the cellular lipid saponifiable and nonsaponifiable fractions were increased 1.5 and 5 fold over those measured when serum was present. Also, HTC cells cultured in media containing lipid poor serum (lacking all lipoproteins with a solvent density less than 1.26 mg/ml) gave a four- to fivefold increase of radioactivity incorporated into the cellular nonsaponifiable fraction. The activities of citrate cleavage enzyme, "malic" enzyme, fatty acid synthetase, malate dehydrogenase, lactate dehydrogenase, glucose-6-P dehydrogenase, 6-P-gluconate dehydrogenase, acetyl CoA carboxylase and pyruvate carboxylase were measured in HTC and HC cells cultured under normal conditions. It was found that HTC cells had two to three times more hexose monophosphate shunt dehydrogeneses and five times more lactate dehydrogenase activity than the HC cells. In general the remaining enzyme activities were not significantly different.

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THE EFFECTS OF ALKALIES ON POLYESTER. J. J. Cramer, W. Lozo and J. D. Ciko, Wyandotte Chemicals Corporation, Wyandotte, Mich. 48192.

Continued emphasis upon the relationship between phosphates and the environment is causing some companies to adjust their detergent formulas with stronger alkaline materials. Consequently, the effects of these stronger alkalies upon modern synthetic fibers is becoming increasingly important. Laboratory tests which study the effects of various alkaline solutions upon Mylar film in a clased system have led to a mathematical formalization of polyester degradation phenomena. The degree to which the polyester structure is damaged may be measured in terms of its per cent weight loss (%W_1) and defined as being directly proportional to the independent variables exposure time (t), temperature (T) and molar hydroxyl ion concentration [OH^-], i.e., f[t, T, (OH^-)] = %W_1. This hypothesis is supported by the agreement found between data obtained from laboratory tests and calculated values based upon equations derived from adaptations of the Box method of experimental design.

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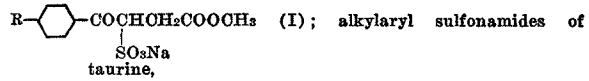
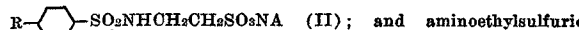
DEVELOPMENT OF A PHOSPHATE FREE HOME LAUNDRY DETERGENT. W. M. Linfield, K. A. Roseman and H. G. Reilich, IIT Research Institute, Chicago, Ill. 60616.

A group of multifunctional surfactants were synthesized in order to arrive at materials which might function well in hard water. We have found the salts of alkylaroyl sulfopropionates,



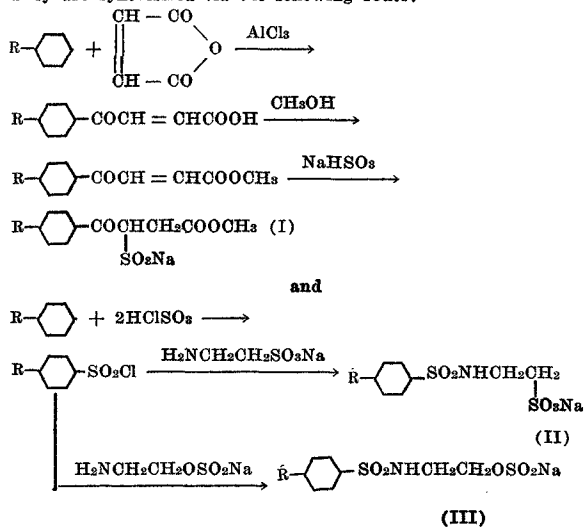
(I); alkylaryl sulfonamides of taurine,



(II); and aminoethylsulfuric acid,

(III) to be quite effective.

They are synthesized via the following route:



These materials were formulated into the detergents with the aid of various organic and inorganic builders other than condensed phosphates. The detergency of these materials was evaluated with the aid of standard test cloths. Analogously formulated detergents based upon LAS were also included in this evaluation study. It was found that a number of satisfactory phosphate-free heavy duty detergent formulations could be developed.

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EFFECT OF WATER HARDNESS AND DETERGENT BUILDERS ON ENZYME ACTIVITY. W. L. Groves, B. W. Terry and R. D. Katstra, Continental Oil Co., Ponca City, Okla. 74601.

Stain removal with alkaline protease enzymes was studied on blood and grass stains. Detergent builder type and amount were varied as well as water hardness. Enzyme response differed between the two stains with only grass being sensitive to the Ca ion-builder ratio. The most critical effect of Ca-builder ratio variations was the change in pH of the wash system, which subsequently had a marked effect on stain removal. At controlled pH conditions, both builder and Ca ions have a negative effect on enzyme activity towards grass stain. The optimum condition for enzyme activity is Ca ions equivalent to the sequestering level of builder. Some other cations in addition to Ca displays this "neutralizing the builder" effect.

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SYNERGISTIC INTERACTIONS OF SHAMPOO INGREDIENTS. Peter M. Hay, Sandoz-Wander, Inc., Hanover, N.J. 07936.

Control of viscosity of a shampoo is usually obtained by adding thickening agents to water solutions of surfactants and other ingredients. The thickening agents may or may not perform other functions, e.g., foam boosting, hair control, etc. This report describes shampoos in which two different surfactants interact synergistically to produce high viscosity water solutions at relatively low total solids without the addition of thickeners. The cleaning ability of two surfactants and their mixtures, measured by determining the grease content of raw wool before and after cleaning with shampoo solution, was proportional to the ratio of ingredients. The viscosity of a mixture of solutions of equal parts of two surfactants was much higher than either surfactant alone, however. Certain additional additives enhanced the viscosity building whereas others decreased it. Surfactant pairs which show synergistic viscosity increase are the following: Sandopan TFL Conc., a sulfonated amide-amine, with sodium lauryl sulfate and Sandopan TFL Conc. with sodium alkylbenzene sulfonate.

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TALLOW ALKANOLAMIDES: PREPARATION AND EFFECT ON SURFACTANT SOLUTIONS. J. K. Weil, N. Parris, W. R. Noble, F. D. Smith and A. J. Stirton, E. Utiliz. Res. Dev. Div., ARS, USDA, Philadelphia, Pa. 19118.

The reaction of one or two moles of epoxide with simple amides gave a low yield of monooxyalkylated products and large amounts of unreacted amide and polyoxyalkylated amides. Oxyalkylated amides containing a more favorable distribution of products were obtained by oxyalkylating hydroxyethyl amides. Reaction products of N-2-hydroxyethyl-amides and N,N-bis-(2-hydroxyethyl)amides with ethylene oxide and propylene oxide were compared. Amides with short oxyalkyl chains and long fatty chains were not water soluble by themselves but were dissolved by aqueous detergent solutions and had characteristic "Krafft Points." The Krafft point was the same for combinations with different soluble detergents and was independent of total concentration, amide to surfactant ratio up to 1:4 and the presence of hard water ions. Solubility of sodium octadecyl sulfate and disodium α-sulfostearate was increased by the presence of alkanolamides. N,N-bis-(2-hydroxyethyl) palmitamide, N,N-bis-(2-hydroxyethyl)stearamide and their oxyalkylated products increased the viscosity of LAS solutions in the same way as diethanolamides of coconut oil. N,N-bis-(2-hydroxyethyl)pelargonamide did not show this effect. One per cent N,N-bis-(2-hydroxyethyl)oleamide and N-[2-(2-hydroxyethoxy)ethyl]-oleamide formed petrolatum-water emulsions which were stable for more than a month. Other oleamides and disubstituted palmitamides and stearamides formed petrolatum and carbon tetrachloride emulsions with limited stability.

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BIODEGRADATION OF SULFATED ALKANOLAMIDES. T. C. Cordon, E. W. Maurer and A. J. Stirton, E. Utiliz. Res. Dev. Div., ARS, USDA, Philadelphia, Pa. 19118.

Biodegradability as determined by various procedures has been studied for sulfated alkanolamides derived from tallow fatty acids. The compounds tested were the sodium salts of sulfated hydroxyethyl palmitamide, sulfated hydroxypropyl stearamide and sulfated 2-(2-hydroxyethoxy)-ethyl stearamide. Sodium hexadecyl sulfate and linear alkylbenzenesulfonate (LAS) were used as reference materials. Aerobic tests were carried out in which the detergents were the sole source of carbon. After 2–3 days sulfated alkanolamides degraded sufficiently to lose their methylene blue activity. Approximately

the same time was required for loss of methylene blue active substance (MBAS) with sodium hexadecyl sulfate, whereas 8–10 days were required for loss of MBAS when LAS was used. Reduction of organic carbon was somewhat slower and less complete after 21 days incubation with the alkanolamides than with sodium hexadecyl sulfate. Sulfate ions were not formed from the alkanolamides until late in the test and then only small amounts were present. In aerobic river water tests of alkanolamides MBAS was lost in two days compared to one day for sodium hexadecyl sulfate. Under conditions of low oxygen concentration (microaerophilic) at room temperature 8–10 days were required for loss of MBAS with the alkanolamides and 9–10 days with sodium hexadecyl sulfate. LAS was not degraded under these conditions. Microaerophilic tests were carried out using known, controlled nutrient salts and the detergents as the sole source of carbon. Sludge from the anaerobic digester of a sewage plant was the inoculum. Loss of MBAS was slower than in the microaerophilic river water tests. Neither the addition of yeast extract to the growth medium nor the activation of the inoculum by growth on an anaerobic nutrient medium increased the rate of biodegradation. When solutions containing 20 ppm of detergent were allowed to percolate through a local soil, no detergent was detected in the effluent from the alkanolamides or sodium hexadecyl sulfate. When LAS was tested, a small amount of detergent was present in the effluent and the quantity increased so that 13% of that added was present after 15 days.

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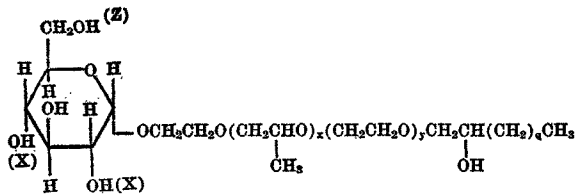
SOME NEW BIODEGRADABLE SURFACTANTS DERIVED FROM CORN STARCH. PETER E. THROCKMORTON, Ashland Chemical Co., Minneapolis, Minn. 55420, DAVID AELONY, RICHARD R. EGAN and F. H. OTEY.

Accumulation of detergents in lakes and streams causes foamy, unpleasant, polluted waters. Possibilities of providing more adequate control of biodegradation of syndets were explored. Polyol glycosides, obtained by reacting starch with either ethylene glycol or glycerol, were used to prepare biodegradable surfactants. Pilot-plant studies demonstrated that the new surfactants are economically feasible. In polyol glycoside mixtures there are several reactive primary hydroxyl sites, as well as the somewhat less reactive secondary sites. To prepare surfactants, these sites were first etherified with ethylene oxide or a mixture of ethylene and propylene oxides. The resulting alkoxylated glycosides were then reacted with long chain fatty materials. A hydrophobic-hydrophilic balance was achieved by varying the number of alkoxide and fatty groups per glycoside molecule until the products demonstrated optimum cleaning and surface active properties. The surface active properties were rapidly rendered inoperative by the bacteria of activated sludge. This exceptional biodegradability is attributed to the glycoside moiety present. It was discovered that the hydrophobic or oleophilic groupings, necessary for surfactancy, could be attached to the alkoxylated glycosides by esterification. Alternatively, by reacting the alkoxylated glycosides wih a fatty alkane-1-oxide to link a fatty group through a β-hydroxy ether, surfactants were made that had superior detergency properties. This reaction of α-olefin oxides and alkoxylated glycosides provides what is believed to be a new concept in surfactant preparation. The general structure illustrates this new concept for biodegradable surfactants. Parameters x, y and q can be varied to change surfactancy, detergency and biodegradability properties.



X and Z are the ethylenoxy and propylenoxy polymer groups terminated by hydroxyl or β-hydroxyalkyl; x, y and q are the integers.

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THE AZURE A METHOD FOR THE ANALYSIS OF SULFATIDES AND THE INFLUENCE OF THE STATE OF VITAMIN A DEFICIENCY ON THE FORMATION OF THIS SULFOLIPID, IN VIVO. E. L. KEAN, Case Western Reserve University, Cleveland, Ohio 44106.

The formation of a colored complex between the thionine dye, azure A, and sulfatide that is extractable by a solution of chloroform-methanol (1:1) is the basis of a sensitive, easily performed nonhydrolytic procedure for the quantitative analysis of sulfatides. With the exception of some of the phospholipids, the reaction is highly specific for the sulfolipids. A wide variety of lipids neither contributed to nor interfered with the formation of color by sulfatide. The molar extinction coefficient for the sulfatideazure A complex is 6.88×10^4. N-deacylated sphingolipids, such as sphingosine, psychosine or lactosyl sphingosine, as well as aliphatic amines of carbon chain length greater than dodecyl inhibit the formation of the complex between azure A and sulfatide, probably by competing with the cationic dye for the anionic lipid. This assay method can be used to measure the sulfatide concentration in tissue extracts. Since some of the phospholipids are reactive, this method is more reliable when used after these materials have been removed, as for example by the use of Florisil chromatography. Contradictory findings have appeared in the literature over the past 16 years or so dealing with the influence of vitamin A deficiency on the processes involved in biological sulfation. The effect of vitamin A deficiency on the content of sulfatides (using the azure A assay) and the incorporation of ^{35}S into sulfatides was studied in young rat brain during the period of rapid myelination in the weanling rat, in the adult rat and in brain and ocular tissue of young rabbits. The techniques of paired or restricted feeding of controls and manipulation of litter sizes (thus influencing body weight) were employed in the present study in order to reduce possible variation due to the effects of malnutrition. No significant differences between deficient and control animals were detected that could be attributed to the nutritional deficiency, per se. It is concluded from these studies that the state of vitamin A deficiency does not interfere in the formation of the sulfated cerebrosides and perhaps of the processes involved in biological sulfation in general.

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UNGULIC ACID AND ITS OCCURRENCE IN ANIMALS. ERKKI LEIKOLA, University of Helsinki, Helsinki, Finland.

Human epidermis, hair and nails, pig bristles, lamb's wool, bovine and horses' hooves, feathers and kidney capsule contain a lipid fraction composed of ceramide, sialic acid, galactose, galactosamine and sulfate in equimolar amounts. Fatty acid analysis showed that stearic acid was the major component, with minor amounts of palmitic and arachidic acids. This ganglioside sulfate is called "ungulic acid" because it was first identified from horses' hooves. Ungulic acid has a molecular weight of 1,280 and a melting point of 190–192 C. It is soluble in methanol and chloroform and slightly soluble in water. In chloroform-methanol-water (24:7:1), ungulic acid has an R_f value of 0.31. The ungulic acid content of various tissues ranges from 0.1% to 0.25% of the fresh weight. Ungulic acid has antibacterial activity against several microorganisms, e.g., against *Staphylococcus aureus, Streptococcus faecalis* and *Mycobacterium tuberculosis*.

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THE HALOSULFOLIPIDS OF *OCHROMONAS DANICA*. T. H. HAINES, City College of City University of New York, New York, N.Y. 10031, MANUEL POUSADA, ALEX BRUCKSTEIN, BHUPESH DAS.

A mixture of alkyl disulfates has been found in *Ochromonas danica* and in its growth medium. The mixture consists of 1,14-dodecyl disulfate, 1,15-tetradecyl disulfate, accompanied by isomers in which 0–6 halogens substitute for hydrogens on these disulfates. The major compound obtained from cells of the fresh water phytoflagellate which are cultured in halide medium (3:8 saline) is the disulfate of 2,2,11,13,15,16-hexahalo docosane diol. Either chloride or bromide is satisfactory. This is accompanied by the disulfates of 2,2,12,14,16,17-hexahalo-1,15-tetracosane diol, 12-C and 14C-pentahalo disulfates, several tetrahalo, trihalo, dihalo and monohalo compounds in both series.

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STRUCTURE OF A GLYCOLIPID SULFATE IN *HALOBACTERIUM CUTIRUBRUM*. MORRIS KATES, University of Ottawa, Ontario, Canada, PAUL DEROO.

Cells of the extremely halophilic bacterium, *Halobacterium cutirubrum*, contain an unusual glycolipid sulfate composed of a sulfated trisaccharide linked glycosidically to di-*O*-phytanyl glycerol. The sulfated trisaccharide consists of glucose, mannose and a terminal galactose-3-sulfate; the glucose is linked at C_2 and mannose at C_6. We report here the elucidation of the complete structure of the sulfated glycolipid. Solvolysis of the ammonium salt of the glycolipid sulfate (TLC R_f 0.1, in $CHCl_3/CH_3OH$/90% HAC, 30:4:20) in tetrahydrofuran in the presence of 0.02 N HCl at room temperature for 30 min resulted in quantitative cleavage of the sulfate ester group, yielding the free triglycosyl diphytanyl ether (R_f 0.34, in $CHCl_3/CH_3OH$/90% HAC, 3:4:20). Hydrolysis of the desulfated glycolipid in chloroform–0.24 N methanolic HCl (4.5:6, v/v) at room temperature for four days resulted in stepwise cleavage of the sugar residues to give a mixture of the mono- and diglycosyl diethers (R_f, 0.94, 0.62, respectively, in $CHCl_3/CH_3OH$/90% HAC, 30:4:20), which were isolated by preparative thin layer chromatography. These were then hydrolyzed separately with methanolic-HCl, followed by aqueous HCl, and the resulting sugars were identified by paper chromatography in pyridine-ethylacetate-water (2:5:5, upper phase). The monoglycosyl diether contained only glucose, and the diglycosyl compound contained both glucose and mannose. The structure of the glycolipid sulfate is SO_4^--3-Gal-(1→6)-Man-(1→2)-Glu-(1→1′)-2′,3′-di-*O*-phytanyl-*sn*-glycerol.

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MYCOBACTERIAL SULFOLIPIDS. M. B. GOREN, National Jewish Hospital, Denver, Colo. 80206.

Virulent strains of *Mycobacterium tuberculosis* elaborate a multitude of glycolipid sulfatides which have been implicated in neutral red fixation, a cytochemical test which distinguishes virulent from avirulent strains. The principal sulfatide class, SL-I, has been identified as a 2,3,6,6′ tetraacyl trehalose-2′-sulfate. With allowance for certain levels of homology within each acyl substituent, in SL-I, the average acyl functions are 1 mole each of palmitic acid and of a heptamethyl-triacontanoic acid, and 2 moles of 17-hydroxyoctamethyldotriacontanoic acid; the extensive methyl branching is accounted for by propionate incorporation onto a palmitate root. A very facile spontaneous desulfation of NH_4SL-I and related substances may define a role for these sulfolipids in the pathogenesis of tuberculosis.

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SUBCELLULAR LOCALIZATION OF FATTY ACID SYNTHESIS IN SEEDS. J. L. HARWOOD and P. K. STUMPF, University of California, Davis, Calif. 95616.

Subcellular fractions of good homogeneity have been separated from seed tissues which have high or low lipid content. The capacity of these fractions to synthesise fatty acids from 1-^{14}C-acetate and 1,3-^{14}C-malonyl CoA was studied and localization of activity determined by careful reference to enzyme markers. The particle free supernatant fraction of seeds synthesises fatty acids from malonyl CoA in the presence of cofactors (NADPH, NADH, GSH and acyl carrier protein). However, a lack of acetyl CoA carboxylase prevented the utilization of acetate as substrate in this fraction from castor bean, lupin and pea seeds. Other particulate organelles also show synthesing ability in different seeds, e.g., the mitochondria of germinating pea and lupin, the microsomes of pea and the fat fraction of developing castor bean. Organelles of each seed produce characteristic fatty acids which are contrasted with the homogenate in vitro and seed in vivo capacities. Thus the capacity and site of synthesis for fatty acids varies in different seeds. Generalizations derived from studies with bacterial or mammalian cells cannot, necessarily, be applied to higher plant systems.

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STIMULATION OF STEAROYL DESATURASE BY *SN*-GLYCERO-3-PHOSPHATE IN MOUSE LIVER MICOSOMES. P. K. RAJU and RAYMOND REISER, Texas A&M University, College Station, Tex. 77843.

A quantitative assay system for the determination of stearoyl desaturase in mouse liver microsomes was developed. Under optimum conditions, microsomes obtained from mice fed a fat free diet had desaturase activity of about 3 mμmoles/min/mg protein, and those fed a stock diet had an activity of about 1.5. *sn*-Glycero-3-phosphate (GP) at 4 mM concentration stimulates the desaturase activity about twofold. This stimulation is very specific for GP since glycero-2-phosphate or glycero-1-phosphate are ineffective. Addition of GP to the incubation mixture increases the amount of esterified fatty acids, mainly in the form of diacyl-glycerophosphoric acid. Studies on the rate of esterification and desaturation under controlled concentrations of GP and NADH show that esterified stearic acid is not desaturated and that the stimulation may be due to esterification of the desaturation product.

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THE EFFECT OF NITRATE AND PHOSPHATE CONCENTRATIONS ON THE FATTY ACID METABOLISM OF THE ALGAE *CHLAMYDOMONAS REINHARDTIS*. J. B. SADDLER, J. B. FAGAN and FRIEDA B. TAUB, University of Washington, Seattle, Wash. 98105.

This study was designed to elucidate the relationships between the nitrate and phosphate concentrations in the growth media and the fatty acid composition of the green algae *Chlamydomonas reinhardtis*. Algae were grown in a defined inorganic media containing graduated initial concentrations of nitrate and phosphate ranging from high (5.0:4.0 mM) to very low (0.025:0.002 mM, nitrate-phosphate). Total cell yield decreased with decreasing concentrations of nitrate and phosphate, but the lipid content of the cells was found to be maximum at the intermediate nitrate and phosphate concentrations of 0.5 mM and 0.04 mM, respectively. The per cent composition of the algal fatty acids was invariable at high and moderate nitrate and phosphate concentrations, but when these concentrations were decreased, there were fundamental alterations in the fatty acid composition. Saturated fatty acids 16:0 and 20:0 decreased from 31% to 23% and from 4% to 2%, respectively, while stearic acid increased from trace amounts at high nitrate and phosphate concentrations to a maximum of 12% at low concentrations. Fatty acids 16:3 and 18:4 decreased in percentage from 4% to less than 1% and from 20% to 3%, respectively, while C-20 and C-22 polyunsaturates increased from trace amounts to a maximum of 21%. Though there were major changes in individual fatty acids, the overall result of these individual changes was to maintain the relative per cents of saturated, monounsaturated and polyunsaturated fatty acids at the constant levels of $41 \pm 5\%$, $20 \pm 3\%$, and $39 \pm 2\%$, respectively.

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FATTY ACIDS OF MOSS AND FERN LIPIDS. W. H. ANDERSON, J. L. GELLERMAN and H. SCHLENK, University of Minnesota, Austin, Minn. 55912.

The fatty acids of moss lipids contain up to 40% 20:4ω6 (arachidonic) and 10% 20:5ω3 acids together with the related dienoic and trienoic and the common more saturated fatty acids. The proportions of those highly unsaturated acids in lipids from *Mnium cuspidatum* are rather constant through the growing season. Greater differences in composition are found in parts of the plant. Rhizoids contain 10%, sphorophytes, 15%, gamotophytes and runners 35% 20:4:ω6 in the lipids. Similarly 20:5ω3 is lowest in the rhizoids. The highly unsaturated fatty acids occur in appreciable amounts, although at different levels, in all lipid classes including the triglycerides. About 25% 11–16:1 acid is in the rhizoid lipids of *M. cuspidatum* but only traces of this unusual structure are found in other parts of the plant. The 11–16:1 acid does not occur in appreciable amounts in fern, genus *Matteucia*. Root, rhizoid and immature leaf lipids of the fern contain 10% 20:4ω6 and very little 20:5ω3 whereas both of these acids are found equally to 5% in the mature leaf lipids. The higher members of the ω3 series apparently do not replace the parent acid 18:3ω3, in these lipids. As in other plants, 18:3ω3 is a major constituent of the lipids in green parts of the fern. However, in the moss, this acid is much less than 20:4ω6.

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DISCRIMINATION OF FATTY ACID ISOMERS BY THE LAYING HEN. T. L. MOUNTS, E. A. EMKEN, W. K. ROHWEDDER and H. J. DUTTON, Northern Regional Research Lab., Peoria, Ill. 61604.

Mixtures of 1-^{14}C-methyl oleate with methyl elaidate-9,10-^{3}H and methyl oleate-9,10-^{3}H with 1-^{14}C-methyl elaidate were force fed in a one shot dose to a laying white Leghorn hen in separate experiments and the eggs were subsequently collected. Lipids extracted from the fifth egg laid after feeding showed the highest level of radioactivity. Neutral lipid and phospholipid fractions isolated from the eggs were radioactive. Gas liquid radiochromatography of the methyl esters prepared from the isolated lipids showed that the ^{14}C and ^{3}H labels were present almost exclusively in the octadecenoic acid fraction. Dual label liquid scintillation assay of the neutral lipids and phospholipids gave ^{3}H/^{14}C ratios which were compared to the ^{3}H/^{14}C ratio of the mixture fed. Evidently the hen partially favors the *cis* isomer during the incorporation of monoene into the neutral lipid and phospholipid of the egg. A mixture of ethyl *trans*-9-octadecenoate and ethyl 9,10-dideutero-*cis*-9-octadecenoate was fed concurrently with a methyl oleate-9,10-^{3}H and 1-^{14}C-methyl elaidate mixture. This simultaneous experiment showed that the labeled *cis*-monoenes were preferentially incorporated into the egg lipid. This discrimination is greater in the phospholipid.

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VARIATIONS IN THE LIPID CONTENT DURING THE METAMORPHOSIS OF *ANTHONOMUS GRANDIS* (COLEOPTERA). R. D. HENSON, A. C. THOMPSON, R. C. GUELDNER and P. A. HEDIN, Boll Weevil Research Lab., ARS, USDA, State College, Miss. 39762.

Changes in the amounts, composition and distribution of phospholipids and neutral lipids during the metamorphosis of the boll weevil, *Anthonomus grandis* Boheman, were investigated. Neutral lipid increased from 76.6% total lipid in the last instar larvae to 81.8% in the early pupal stage while the phospholipid decreased by 5.2% across the same interval. Fatty acid composition of the total lipid was constant throughout the three stages analyzed. However, investigation of neutral and polar fractions revealed a concentration of linoleic acid in the phospholipids, while palmitic and oleic acids comprised the bulk of fatty acids in the neutral lipids. Phosphorus and fatty acids analysis of the individual phospholipids revealed a unique situation in which phosphatidyl ethanolamine predominated over phosphatidyl choline. This situation does not exist in any other stage of the development of this insect, including the egg. Other phospholipids identified and analyzed for fatty acids were lysophosphatidyl choline, sphingomyelin, phosphatidyl inositol, phosphatidyl serine and polyglycerophosphatide.

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EFFECT OF MATURITY ON THE FATTY ACID COMPOSITION OF EIGHT VARIETIES OF PEANUTS GROWN AT PERKINS, OKLAHOMA IN 1968. C. T. YOUNG, Georgia Experiment Station, Athens, Ga. 30601, M. E. MASON, R. S. MATLOCK and G. R. WALLER.

The primary purpose of this study was to examine the influence of variety and maturity on the fatty acid composition of the oil of peanuts grown under essentially normal but measured field conditions. The stability of peanut oil, as measured by the oleic acid-linoleic acid (O/L) ratio, was also examined. Eight peanut varieties (*Arachis hypogaea* L.) of highly homozygous breeding lines were grown at Perkins, Oklahoma, in 1968. The peanuts were harvested, dried at 90 F, classified into maturity levels and stored at —20 C to minimize chemical changes, particularly in the free amino acids. Oils were extracted with diethyl ether. Methyl esters were prepared by the method of Jellum and Worthington (1966) and analyzed by gas liquid chromatography on a DEGS ¼ in., 6 ft glass column. Fatty acid composition was determined by normalization of peak areas and the values reported are therefore relative proportions of total fatty acids analyzed by this method. The fatty acid compositions of three maturity groups for eight varieties with five different harvest dates are reported. Mature peanuts usually contained more stearic (18:0) and oleic (18:1) acids and less linoleic acid (18:2) and other fatty acids. Behenic (22:0) and arachidic (20:0), which were recently implicated in heart disease, were lower in the mature nuts. O/L ratios, which indicate longer oil stability, were higher in mature peanuts within each variety.

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THE EFFECT OF ETHIONINE ON UNSATURATED FATTY ACID SYNTHESIS IN THE LIVER. L. A. WITTING, Elgin State Hospital, Elgin, Ill. 60120.

In agreement with the observations of Lyman et al. (1969 and 1970), the hepatotoxin, ethionine, was found to greatly depress the hepatic synthesis of unsaturated fatty acids in male rats prior to fatty liver induction. However, as relatively low levels of neutral lipid accumulated in the liver, a progressive increase in the incorporation of ^{14}C labeled acetate into unsaturated fatty acids was observed. Increased incorporation of the labeled acetate reached values 3–5 times the control level and appeared to represent an actual increase in synthetic activity, since significant net changes in hepatic phospholipid fatty acid composition were detected. This response was significantly moderated by relatively large doses of α-tocopherol and partially moderated by N,N′-diphenylparaphenylenediamine. Inclusion of polyunsaturated fatty acids in the diet decreased the accumulation of hepatic neutral lipids and extended the period of depressed enzymic activity. This initial depression of unsaturated fatty acid synthesis did not appear to be greatly effected by α-tocopherol in N,N′-diphenylparaphenylenediamine.

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THE CHOLESTEROL-RICH VERY LOW DENSITY LIPOPROTEINS OF BROAD-β DISEASE (TYPE III HYPERLIPOPROTEINEMIA): RELATIONSHIP BETWEEN THE ABNORMALITIES OF LIPID COMPOSITION AND ELECTROPHORETIC MOBILITY. W. R. HAZZARD and E. L. BIERMAN, V.A. Hospital, Seattle, Wash. 98108.

Two abnormal properties of isolated very low density lipoproteins (VLDL) ($d < 1.006$) in broad-β disease, slow electrophoretic mobility and triglyceride (TG) poor, cholesterol (C) rich composition, have been utilized to segregate this disorder from endogenous lipemia (Type IV). To determine the interrelationship of these properties and whether they are abnormal in a qualitative or quantitative sense, VLDL subfractions (S_f 100–400, 60–100, 30–60, 20–30) were isolated from subjects with broad-β disease ($n = 5$) and with endogenous lipemia ($n = 6$) by density gradient ultracentrifugation and analyzed after starch block electrophoresis. In both disorders, a continuum of decreasing mobility and increasing C-content (with reciprocally reduced TG) was recorded as a function of declining S_f rate, the proportion of cholesterol ester increasing pari passu. In all instances changes in lipid composition paralleled those in electrophoretic mobility. However, in broad-β disease, each subfraction from S_f400 to S_f30 was, as a whole, more C rich and slower in mobility than in endogenous lipemia, more closely resembling the next lower S_f subclass from Type IV (e.g., S_f100–400 in Type III was comparable to S_f 60–100 in Type IV). Nevertheless, within each subfraction, a small proportion of lipoproteins in Type IV exhibited identical lipid composition and electrophoretic mobility to those in Type III, and the S_f 20–30 subfractions were identical in both disorders [β-migrating, (0.4 relative to albumin) and C rich (TG/C = 1)]. Thus these findings suggest that the abnormal properties of VLDL lipid composition and electrophoretic mobility characteristic of broad-β disease may both arise through a common process, one which may not be unique to this disorder.

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CHOLESTEROL ESTERS AND THE LESIONS OF ARTERIOSCLEROSIS IN MAN. WILLIAM INSULL, JR., Hahnemann Hospital, Philadelphia, Pa. 19102, P. DIETER LANG, YOSHIYA HATA and JOHN HOWER.

The dominant lipids accumulating in the lesions of arteriosclerosis in man are cholesterol esters. The prominence of cholesterol oleate in early lesions and of cholesterol linoleate in later lesions has become established in the past decade. However, knowledge of the form of the lipids in the tissues is limited and knowledge of mechanisms responsible for its accumulation is unknown. Studies in this laboratory demonstrate

that the cholesterol esters in the early lesions, the aortic fatty streak, occur largely as spherical anisotropic droplets about 2 μ in diameter containing 95% of the lipid as cholesterol esters, 50% of which is cholesterol oleate. The droplets have optical properties of a radially symmetical crystalline structure, probably that of liquid crystals. Studies of their fine morphology with the scanning electron microscope indicate a basic spherical shape with variations in size, surface texture and shape. Comparison of droplets from early and late lesions appear to show related differences in the fine morphology. The unique chemical composition and physical forms of these lipids suggest that mechanisms highly selective for lipid composition and possible directive for structure may be responsible for accumulation of these droplets in the arterial tissues during the pathogenesis of arteriosclerosis.

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CHOLESTERYL ESTER METABOLISM IN NORMAL AND ATHEROSCLEROTIC ARTERIAL TISSUE. R. W. St. Clair and H. B. Lofland, Jr., Bowman Gray School of Medicine, Winston-Salem, N.C. 27103.

The accumulation of cholesteryl esters in the arterial wall is a hallmark of atherosclerosis. Unlike free cholesterol, which appears to arise almost exclusively from the blood, cholesteryl esters are synthesized, mainly by esterification of preexisting cholesterol, in substantial amounts in atherosclerotic arteries. Esterification of cholesterol by aortas from White Carneau pigeons was studied using a cell free preparation (1000XG supernatant fraction) and intact tissues (organ culture and arterial perfusion). More than 90% of the fatty acids synthesized from 1-^{14}C-acetate was found esterified to complex lipids. In normal arterial tissue more than 98% of these fatty acids was esterified to triglyceride and phospholipid, in severely atherosclerotic vessels, as much as 50% of the newly-synthesized fatty acids was esterified to cholesterol. Oleic acid was the major newly-synthesized fatty acid esterified to cholesterol. Esterification of fatty acids to cholesterol required ATP and CoA with more than 80% of the esterifying activity located in the particulate fraction (105,000XG pellet). Cholesterol was esterified by a mechanism similar to that described for liver and adrenal (fatty acyl-CoA-cholesterol acyl transferase). We could not show esterification of cholesterol using, as substrate, lecithin labeled at the 2 position with 1-^{14}C-oleic acid. In cell-free preparations, added radioactive cholesterol was not esterified even though fatty acids were readily esterified to cholesterol preexisting in the artery. On the other hand, using the organ culture technique, it was possible to demonstrate esterification of added radioactive cholesterol and to show a more active esterification in diseased versus normal arteries. Stimulation in esterification of 1-^{14}C-oleic acid to cholesterol was detected after only two weeks of cholesterol feeding (before gross lesions were seen) and reached a maximum increase of 30- to 50-fold after eight months of cholesterol feeding. This stimulation in cholesterol esterification is, to our knowledge, the earliest change in lipid metabolism that has been reported associated with the development of atherosclerosis.

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CHOLESTERYL ESTER METABOLISM IN ARTERIAL TISSUE AND ATHEROMATA. Seymour Dayton and Sam Hashimoto, VA Hospital, Los Angeles, Calif. 90073.

Employing 1-^{14}C-palmityl CoA as substrate, pathway of cholesterol esterification in homogenates of rabbit aorta was studied. Rate of esterification in homogenates of atherosclerotic aorta (i.e., after three to five months of cholesterol feeding) was 28 times the rate observed in homogenates of normal aorta. In both preparations, specific activity of cholesteryl ester and of phosphatide reached plateau levels after ½ hr. At the plateau level, specific activity of cholesteryl ester fatty acid was much lower than specific activity in the β acyl group of lecithin. Occurrence of esterification of cholesterol upon addition of palmityl CoA, and its termination after exhaustion of the substrate, indicate that cholesterol esterification is accomplished by fatty acyl CoA-cholesterol acyltransferase, rather than by lecithin-cholesterol acyltransferase. Other experiments tested the manner in which acceleration of cholesteryl ester synthesis in atherosclerotic aorta is mediated. Particulate fractions (microsomes plus mitochondria) from atherosclerotic and from normal tissues were suspended in supernatants from the same tissues, in all four possible combinations. Cholesterol esterification by particulate material from atherosclerotic aorta was not depressed by incubation with supernatant from normal aorta. Esterification by normal particulate was enhanced slightly by supernatant derived from atherosclerotic aortic homogenate, but this increase was due to esterifying activity present in the supernatant. Thus there was no evidence of a soluble activator in atherosclerotic aorta, nor of a soluble inhibitor in normal aorta. In normal aorta, microsomes contained almost all the cholesterol esterifying activity of the particulate fraction; in atherosclerotic aorta, about 70%.

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CHOLESTERYL ESTERS: THEORETICAL REFLECTIONS. David Kritchevsky, Wistar Institute, Philadelphia, Pa. 19104.

Abstract not available at press time.

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4-^{14}C-CHOLESTEROL DISTRIBUTION IN TISSUES OF RATS FED CORN OIL, BUTTERFAT AND CORN OIL OR BUTTERFAT PLUS CHOLESTEROL DIETS. Anahid Crecelius and R. B. Alfin-Slater, University of California, Los Angeles, Calif. 90052.

A study was made of the distribution of labeled cholesterol in tissues of rats, including the carcass. The animals were fed corn oil or butterfat diets and corn oil or butterfat and 1% cholesterol diets for 15 days prior to the oral administration of the one dose of labeled cholesterol. A comparison of total counts recovered from each group showed considerable loss of counts in the butterfat-fed groups, indicating possible sterol nucleus degradation. Animals fed corn oil had higher levels of radioactivity in tissue cholesterol, which may be attributed to more rapid and better absorption of cholesterol with corn oil or to a slower turnover rate of the absorbed labeled cholesterol. In these animals, approximately 40% of the label was found in the intestinal contents and feces as compared to the 60% of the label found in the above mentioned tissues in the butterfat-fed group. In most of the tissues examined, dietary cholesterol did not greatly influence the per cent of the labeled cholesterol in the tissue cholesterol with the exceptions of liver, intestine and feces in the corn oil fed rats, and intestive, intestinal contents and feces in the butterfat-fed rats. The liver was the chief tissue with increased levels of the label in the saponifiable fraction when cholesterol was present in the diet with either corn oil or butterfat.

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THE METABOLISM OF FATTY ACIDS AND FATTY ALCOHOLS IN FIBROBLASTS (L-M CELLS) GROWN AS MONOLAYERS. M. L. Blank, E. A. Cress and Fred Snyder, Oak Ridge Associated Universities, Oak Ridge, Tenn. 37830.

Esterified and ether-linked aliphatic moieties of glycerolipids are derived from fatty acid and fatty alcohol precursors, respectively. Recently, the interconversion of these precursors has been demonstrated. Fibroblasts (L-M cells) grown in suspension cultures contain significant quantities of ether-linked lipids; under these conditions the cells utilize long chain fatty alcohols as the source of the *O*-alkyl chains. In contrast, only minute quantities of ether-linked lipids are synthesized when the same cells are grown as monolayers. The monolayers incorporated both fatty acids and fatty alcohols into phosphoglycerides to about the same extent (>90%), the alcohols being oxidized first to the corresponding acid.

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PHOSPHOLIPID METABOLISM IN CELLS IN CULTURE. W. E. Cornatzer, S. S. Tsao and D. J. Rytter, University of North Dakota Medical School, Grand Forks, N.D. 58201.

Phospholipid composition and synthesis of HeLa and KB tissue culture cells have been studied. The per cent of the total lipid phosphorus of the individual phospholipids of subcellular fractions of HeLa and KB have been determined. Phosphatidyl choline and phosphatidyl ethanolamine are the major phospholipids in the subcellular fractions of the tissue culture cell lines studied. A time study of incorporation of $^{32}PO_4$ into the cellular phospholipids showed phosphatidyl inositol > polyglycerol phosphatide > phosphatidyl ethanolamine > phosphatidylcholine > sphingomyelin and phosphatidyl serine. The time study showed a lag period of 1 to 2 hr for the $^{32}PO_4$ incorporation into choline and ethanolamine phospholipids. Phosphatidyl choline of HeLa, KB cells and Ehrlich ascites tumor were fractionated into four different lecithins by thin layer chromatography. Of the total lecithin phosphorus 82.8% was found in fraction 3 of HeLa cells. The major phosphatidyl cholines found in KB and Ehrlich Ascites tumor was in fractions 3 and 4 and representing 66.8% and 87% of the total lecithin P, respectively. A time uptake of 1,2-^{14}C-choline and 1,2-^{14}C-ethanolamine into the four lecithin fractions in Ehrlich Ascites tumor cells was investigated to determine the two biosynthetic pathways of lecithin synthesis. The incorporation of 1,2-^{14}C-choline into the lecithin fractions 3 and 4 was 100 times that of the 1,2-^{14}C-ethanolamine. These studies give evidence that the CDP choline-diglyceride pathway of lecithin provides most of the lecithin found in the Ehrlich Ascites cells. The enzymatic activity of phosphatidylmethyltransferase activity was very low in HeLa cells.

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STEROL FLUX AND SYNTHESIS IN TISSUE CULTURE CELLS. G. H. Rothblat, The Wistar Institute, Philadelphia, Pa. 19104.

Studies have been conducted on the metabolism of unesterified sterol by tissue culture cells grown in sterol free medium. These investigations have demonstrated that both the influx and efflux of sterol is a function of the concentration of protein, phospholipid and sterol present in the culture medium. Measurements of sterol synthesis in cells grown under conditions in which sterol flux is varied indicate that cellular synthesisis directly related to internal cellular sterol concentrations. Increased influx of exogenous sterol results in reduced synthesis; increased efflux promotes de novo synthesis as cells attempt to replace sterol lost to the culture medium. Other experiments utilizing a number of different steroids have determined which steroids are capable of eliciting a feedback response on de novo sterol synthesis. Steroids such as cholesterol, desmosterol, lathosterol and 7-dehydrocholesterol will, when added to the culture medium, inhibit cellular sterol biosynthesis without producing pronounced cellular toxicity. Other compounds such as coprostanol and cholest-4-en-3-one also reduce cellular sterol synthesis but also inhibit cell growth. Cholestanol and the C_{28} and C_{29} phytosterols have no marked effect on either synthesis or growth. Additional studies on the incorporation and metabolism of some of these steroids have been conducted in an attempt to gain information on the mechanisms involved in sterol feedback in tissue culture cells.

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GLYCOSPHINGOLIPID METABOLISM IN FIBROBLASTS CULTURED FROM PATIENTS WITH INBORN ERRORS OF METABOLISM. Glyn Dawson and Reuben Matalon, University of Chicago, Chicago, Ill. 60637.

Fibroblasts cultured from human skin or bone marrow contain glycosylceramide (GL-1a), galactosyleeramide (GL-1b), lactosylceramide (GL-2a), trihexosylceramide (GL-3a), globoside, G_{M3} and G_{D3}. Traces of sulfatides, G_{M1} and G_{D1a} gangliosides may be found in normal cells but we have only found them in significant amounts in fibroblasts from a patient with a variant of the Hurler syndrome. All glycosphingolipids were estimated as their trimethylsilyl methylglycosides by gas liquid chromatography and, where necessary, their structures were proved by direct mass spectrometry. Reproducible analyses can be obtained if the medium is kept constant and the cells are harvested at the same stage of growth. In vivo studies, using U-^{14}C-glucose as the isotopic precursor, cannot be carried out on humans but fibroblasts rapidly incorporate label into the hexose, fatty acid and sphingosine moieties of glycosphingolipids. Since inborn enzymic defects may be demonstrated in cultured fibroblasts, we have used this approach to confirm the defect of GL-1a catabolism in Gaucher's disease and GL-3a catabolism in Fabry's disease. Fibroblasts from patients with Gaucher's disease, lactosylceramidosis and Fabry's disease accumulate GL-1a, GL-2a and GL-3a, respectively, but we have

not been able to consistently demonstrate G_{M2} in Tay-Sachs fibroblasts nor GL-1b accumulation in Krabbe's leukodystrophy fibroblasts. We have found GL-1b accumulation in fibroblasts from one retarded patient, clinically distinct from Krabbe's disease, enabling us to study the in vivo metabolism of a glycosphingolipid which is more characteristic of neural tissue. Since fibroblasts from patients with inborn errors of lipid metabolism show mucopolysaccharide abnormalities we have also studied a number of mucopolysaccharidoses; their glycosphingolipid composition will be discussed. Fatty acid analyses of glycosphingolipids from fibroblasts have provided some evidence for their direct interconversion but the actual composition appears to vary in different disease states.

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PEPTIDE ACTIVATION OF FAT EMULSIONS FOR INTERACTION WITH LIPOPROTEIN LIPASE.

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Two small apoprotein subunits of human plasma lipoproteins have been found to promote formation of an enzyme-substrate complex between lipoprotein lipase and phospholipid-stabilized fat emulsions. One, characterized by carboxyl-terminal alanine, has lower and more variable potency. In plasma from normolipidemic individuals, the two peptides constitute a substantial fraction of the total apoprotein of very low density lipoproteins and a much smaller fraction of that of high density lipoproteins. They also are major components of the abnormal lipoprotein that accumulates in blood plasma during cholestasis. Peptides with the property of activating fat emulsions for interaction with lipoprotein lipase are present in lipoproteins of many mammalian species, but are almost absent from plasma lipoproteins of adult guinea pigs, which contain very little high density lipoprotein. Evidence has been obtained that these peptides have affinity for certain phospholipid rich surfaces. It is suggested that high density lipoproteins constitute a reservoir from which the active peptides can be transferred to newly secreted triglyceride rich lipoproteins and that the peptides may be transferred back to high density lipoproteins following lipoprotein lipase-mediated removal of triglycerides.

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ROLE OF HDL PROTEIN IN LIPOPROTEIN LIPASE ACTIVITY.

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Lipoprotein lipase (LPL) isolated from human post heparin plasma retains triglyceride, diglyceride and monoglyceride hydrolase activities against native lipoproteins or artificial lipid emulsions. Only triglyceride hydrolase activity requires the presence of a lipoprotein protein "cofactor." Evidence to be presented will suggest that such cofactor activity is related to the substrate-binding capacity of the proteins. LPL activity is inhibited by concentrations of either phospholipid or free cholesterol beyond those of the substrate lipoproteins. Evidence from model systems containing purified LPL and purified lecithin-cholesterol acyl transferase suggests that the maintenance of lipoprotein nonsubstrate lipid concentrations may play an important role in the maintenance of LPL activity.

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THE ROLE OF HEPARIN AS A POSSIBLE ALLOSTERIC MODIFIER OF LIPOPROTEIN LIPASE.

J. M. FELTS, University of Toronto, Canada, and T. F. WHAYNE, JR.

In 1953, Brown, Boyle and Anfinsen found that lipoprotein lipase (LPL) activity in postheparin (PH) serum from dogs was inhibited by the in vitro addition of heparin. Korn (1955) found that heart LPL activity was stimulated by heparin. He also noted that triglyceride emulsions had to be "activated" with serum lipoproteins before hydrolysis would occur. To resolve these conflicting observations on the effect of heparin, we carried out a study of the effects of in vitro heparin on PH LPL activity from six mammalian species using a triglyceride emulsion as substrate. In five species, heparin inhibited activity. However, in the rat, heparin stimulated activity. When rat serum was added to PH serum from the other species, in vitro heparin produced a stimulation of activity. These results suggested that rat serum contained a component responsible for the heparin effect. The effects of lipoprotein fractions from rat serum on LPL activity in guinea pig PH serum were examined. Rat high density lipoproteins (HDL) were the most potent activators of this system. We then studied the interaction between HDL and heparin on LPL activity. PH serum from guinea pigs was incubated in an assay system containing triglyceride emulsions (15 mg). In the absence of in vitro heparin, and in the presence of increasing concentrations of HDL, LPL activity followed a hyperbolic curve. When these data were plotted according to Lineweaver-Burk, a straight line was obtained. In the presence of heparin and in the presence of increasing concentrations of HDL, an S-shaped curve was obtained. When these data were plotted according to the linear form of the Hill equation, a straight line was obtained. These data suggest that LPL is a regulatable enzyme and that heparin may function as a specific allosteric modifier. It is possible that an interplay between specific serum activators, which apparently determine the availability of substrate, and heparin, which can modify the enzyme kinetics, may function in vivo to regulate the rate of clearance of triglycerides from serum.

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CLINICAL AND BIOCHEMICAL DIFFERENTIATION OF POSTHEPARIN LIPOLYTIC ACTIVITY.

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The heterogeneity of plasma postheparin lipolytic activity (PHLA) has been established previously. Lipoprotein lipase (LPL) may be distinguished from other triglyceride lipases by its response to known activators and inhibitors. In addition, the identity of the specific lipoprotein activator of LPL has been established and the nature of its effect has been evaluated in greater detail. These observations have been applied to measurement of LPL activity in postheparin plasma from normals and from patients with different types of hyperlipoproteinemia in the studies to be presented.

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COMPARISON OF POSTHEPARIN LIPOLYTIC ACTIVITIES WITH TRIGLYCERIDE, MONOGLYCERIDE, AND PHOSPHOLIPID SUBSTRATES IN NORMAL AND HYPERTRIGLYCERIDEMIC SUBJECTS.

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To test for physiological evidence of separate and specific postheparin lipolytic activity (PHLA) with triglyceride, monoglyceride or phospholipid substrates, four assay procedures were used. Coconut oil (Ediol) was used as the substrate with albumin [TG(Alb)] and with calcium [TG(Ca)] as the fatty acid acceptor in assays for triglyceridase activity. Monoolein substrate was solubilized in desoxycholate for monoglyceridase assay (MG) and phosphatidyl ethanolamine was employed as the substrate for phospholipase assay (PE). PHLA measurements in all four assays were compared in normal adult males and in hypertriglyceridemic subjects with TG (Alb) and/or TG (Ca) below the normal range (uncontrolled diabetes, hypothyroidism, exogenous lipemia). Plasma was obtained after both low (700 units) and high (10,000 units) doses of intravenous heparin. In normal subjects, TG (Alb) correlated closely with TG (Ca) ($r = .91$) and each was significantly related to MG ($r = .91$; .98) and to PE ($r = .91$; .95) ($n = 17$). Subjects with low triglyceridase activity also showed a significant correlation between triglyceridase and phospholipase activities [TG (Alb) vs PE: $r = .46$, $p < .05$; TG (Ca) vs PE: $r = .61$, $p < .01$] ($n = 22$). However, the normal relation between monoglyceridase and either TG (Alb), TG (Ca) or PE was not maintained in the hypertriglyceridemic group. Although there was an overall decrease in MG this was significantly less than the decrease in either TG (Ca) or PE. Thus, in normal subjects, postheparin triglyceridase, monoglyceridase and phospholipase activities are closely related. In subjects with low triglyceridase activity, there appears to be little evidence for separate and specific phospholipase activity since a parallel decrease was observed. These activties may be distinguished from monoglyceridase activity which remains in the normal range in some of the deficient subjects tested.

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ACTIVATION OF LIPOPROTEIN LIPASE: AN EVALUATION OF CALCIUM AS A COFACTOR.

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Lipoprotein lipase (LPL) from rat heart muscle will hydrolyze an artificial triglyceride (TG) emulsion only if the emulsion is converted to an active lipoprotein substrate by the addition of specific serum lipoprotein peptides. In addition, heparin has a marked influence on the rate of hydrolysis of the activated substrate. Cofactors other than serum lipoproteins and heparin may also influence the reaction. In 1955, Korn and Quigley suggested that optimum enzyme activity was also dependent on the presence of either NH_4^+, Ca^{++} or other divalent cations. Using rat heart acetone powders as an LPL source and an activated coconut oil emulsion as substrate, they found that LPL activity was optimal when Ca^{++} was present at a concentration of 10 μmoles/ml or when NH_4^+ was present at a concentration of 50 μmoles/ml. We have reevaluated the Ca^{++} and NH_4^+ requirements of a soluble LPL prepared from acetone powders of rat heart. The LPL assay contained 1.0 ml of enzyme preparation, tris buffer, albumin, TG (Intralipid) and other additions in a final volume of 7.0 ml. One unit of LPL activity equals 1.0 μmole FFA released per hour. Addition of 0.4 ml rat serum to the assay system produced high LPL activity (6.8 units). Addition of purified high density lipoproteins (HDL) from 0.4 ml rat serum produced low activity (1.9 units); however, the addition of Ca^{++} (final conc. 2.5 μmoles/ml) to this system increased activity to 5.9 units. Addition of 2 μmoles of EGTA, a specific Ca^{++} chelating agent, to the system containing serum suppressed LPL activity to 2.8 units; however, addition of 2 μmole Ca^{++} to this system restored LPL activity to 7.3 units. In another study, addition of NH_4Cl buffer, pH 8.6, to a final concentration of 50 μmoles/ml (the optimum NH_4^+ concentration reported by Korn and Quigley) in the assay containing 0.4 ml serum suppressed LPL activity from 8.5 to 2.7 units. EGTA addition to the assay containing NH_4Cl further reduced LPL activity to 0.46 units. We conclude that Ca^{++} has a cofactor function in LPL activity and that the optimum hydrolytic rate of LPL is attained in the presence of physiological concentrations of this cation. We suggest that any assay for LPL activity would be best carried out with a Ca^{++} concentration in the assay system in the range of 1.25–2.5 μmoles/ml so that the non Ca^{++} dependent hydrolase activity is not measured selectively.

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COMMODITY PRICES AS RELATED TO VEGETABLE OIL MARKETING, PRICES AND UTILIZATION.

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Vegetable oil price is principally dependent upon international influences. The supply and demand balances of various vegetable oils in world trade form the basic framework of price level. Even though there is not total flexibility of substitution between oils and fats, there is enough to have a chain reaction effect on prices. Within the United States, soybean oil, cottonseed oil and lard are the principal factors. Soybean oil price has numerous dominant influences other than world demand. One is the supply and availability of beans, which are closely related to government farm programs and support price. Another is demand for soybean meal, which at times may be more or less in balance with demand for oil, so that soybean crush will be predominantly for one or the other resulting in a seesaw effect on price of the other. Another is the government purchase program for salad oil and shortening for domestic and foreign donation programs. Another is the production of cottonseed oil and lard which are by products of the production of cotton and hogs. Consequently they are inherently different than soybean oil which is more nearly a primary product. Profit returns to the crushing industry have a bearing on output of soybean oil. The price relationship available in futures market transactions forms an important part of the profit equation. Changes in supply and demand are quickly translated into price determination via futures, frequently before the changes actually occur. Sometimes this anticipatory reaction in price precludes the actual change in supply and/or demand from taking place. This important pricing function provides those who participate with a strategic tool for more effective corporate planning.

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CONSUMPTION PATTERNS AND TRENDS FOR FATS AND OILS IN THE UNITED STATES.

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Sharp changes have occurred in the consumption of fats and oils in the United States over the past 20 years, both in types of fats and oils utilized and as end products. Along with these trends, per capita use has increased from 67 lb. to over 80 lb. About two thirds are consumed in food products as butter, cooking and salad oils, lard, margarine and shortening. The remaining third is soap, drying oil products, fatty acids, animal feeds and other industrial products. Substitution has continually taken place among the major food fat products as well as between the kinds of fats and oils used in manufacture. During this evolution the trend has been from solid fats to liquids and from animal fats to vegetable fats. Domestic consumption of butter and lard (direct use) has trended downward, but this has been more than offset by expanded use of margarine, shortening and salad and cooking oils. Soybean oil is the major vegetable oil for food fat products and its sharply increased use is largely responsible for an overall increase in per capita consumption, especially salad and cooking oils. This partly reflects the rapid growth in the fast-food industry and rising use of snack type foods.

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NEW MARKETS FOR TALLOW THROUGH RESEARCH. D. M. DOTY, Fats and Proteins Research Foundation, Des Plaines, Ill. 60018, and W. J. SHEPPARD.

Approximately 2.5 million tons of inedible rendered animal fats are produced in the United States annually. These fats compete on the world market with all other fats and oils and with petroleum-derived products that may have a more stable price structure. To assure a continuing profitable market for these animal by-product materials, it is essential that expanded uses be developed for these fats. Research to develop these new uses must be accompanied by careful market analysis and cost of production estimates at every stage of the research and development program. Also, the studies must be so oriented that every advantage can be taken of the unique chemical and physical nature of the fats. To develop a large market volume, the total cost of production must be borne in mind throughout the research program and requires low costs for chemicals and processing. Using this approach, some modest success has been achieved through research supported by the Fats and Proteins Research Foundation at Battelle Memorial Institute and other research organizations. Products derived from fats include a water repellent coating for concrete, an air-entraining agent for concrete, an additive mixture to increase strength and improve water repellency of concrete products, and fat-coated urea for use in livestock feeding. The market potential for animal fats for these products and for other uses will be discussed.

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ECONOMICS OF SUNFLOWER OIL PRODUCTION AND USE IN THE UNITED STATES. W. K. TROTTER and W. D. GIVAN, Richard B. Russell Agricultural Research Center, Athens, Ga. 30604.

U.S. consumption of fats and oils over the past decade increased at an annual rate of 2.9% and reached an all time high of 17 billion pounds in 1969. Exports during this period increased by 7.4% annually and reached 7.4 billion pounds in 1968. About two thirds of U.S. consumption is in food products. Salad and cooking oils, shortening and margarine account for the major share of food oil use. Sunflower oil is well suited for these uses, especially for salad and cooking oils, as is margarine, due to its stable qualities and high ratio of polyunsaturated fatty acids to saturated fatty acids. Sunflower acreage has remained limited due largely to production problems and the inability of the open-pollinated varieties to produce returns that effectively compete for production resources with established crops. Yields of 1,500 to 2,000 lb/acre, with a price of four cents a pound or better for the seed, should permit sunflowers to compete with corn, soybeans, wheat and sorghum in a number of farming areas. The hybrid sunflowers now under development along with improved cultural practices should boost yields sufficiently to make the crop competitive in many areas.

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COST ANALYSES FOR NEW PRODUCTS AND PROCESSES DEVELOPED IN USDA LABORATORIES. V. E. SOHNS, Northern Regional Research Lab., Peoria, Ill. 61604.

Cost analyses frequently implement the research and development program on new products and processes from farm crops. Having cost data available encourages early adoption and application by industry of products and processes that are developed. Furthermore, cost estimates provide information that can serve as a guideline for developing an effective plan of work for research projects. The procedures used to prepare and compile cost estimates at the Northern Regional Research Laboratory follow those generally recommended by most cost engineers for such estimates with perhaps some modifications to fit particular situations. Such process data as raw material requirements, yields of products, general operating conditions, along with other vital process information, are essential for the preparation of an estimate. Accuracy of an estimate depends upon the quantity and quality of the process data made available to the cost engineer. "Order of magnitude" estimates prepared shortly after studies on a process are initiated on a laboratory scale and preliminary cost estimates from process engineering studies in the pilot plant have both found application in research investigations. Several typical examples of cost estimates for new products and processes are the production of fatty acids and nylons from vegetable oils. Literature useful to the cost engineer has been compiled and is cited.

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PATHWAYS OF PLACENTAL FATTY ACID METABOLISM. A. F. ROBERTSON and WARREN KARP, Ohio State University Hospitals, Columbus, Ohio 43216.

Maternal and fetal blood are dissimilar in their fatty acid composition. These differences may arise from the specific placental metabolism of fatty acids, from selective transport or selective oxidation. Cholesterol and lysolecithin possibly derived from the maternal circulation are acylated in placental tissue. These reactions are preferential for the more unsaturated fatty acids. Lecithin and cephalin are synthesized de novo from diglyceride and the respective cytidine diphosphate precursors. These synthetic pathways may explain the placental fatty acid pattern but the apparent absence of hydrolase enzymes rules out their importance in maternal to fetal transport. Transport across the placenta as free fatty acids occurs in other primates. The influence of an intracellular carrier protein or transport as an acyl-carnitine ester is not yet determined. In lower animals free fatty acid transport is not preferential for specific fatty acids. Fatty acid oxidation within placental tissue may alter the pattern of transport. Oxidation of long chain fatty acids is probably preceded by the formation of acyl-carnitine. Approximately 2% of the normal placental O_2 consumption may be due to fatty acid oxidation. The rate of placental fatty acid oxidation is affected by various heavy metals and by polychlorinated biphenyls. Since intrauterine fetal growth is dependent upon the placental transport of essential fatty acids, the clarification of the mode of transport and factors affecting it is quite important.

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THE METABOLISM OF LIPIDS IN THE PLACENTA AND THE FETUS. J. F. ROUX and TAMOTSU YOSHIOKA, Cleveland General Metropolitan Hospital, Cleveland, Ohio 44101.

Lipid concentration, composition, synthesis and breakdown were measured in the brain, lung, liver and placenta of the human fetus at various periods of development. Triacylglycerols (TG), phospholipids (PL), sterols (ST) and sterol esters (SE) were separated by liquid column or thin layer chromatography. The concentration of each fraction was determined spectrophotometrically and their fatty acid composition was identified by gas liquid chromatography of their methylesters. The data show that the lipid concentration per gram tissue wet weight and the tissue composition of each lipid fraction does not change significantly between the 8th and 32nd week of gestation within each organ. PL and ST are the major components of brain and liver tissue, whereas TG are the major constituents of fetal liver and adipose tissue. All fetal tissues investigated contained 11 to 18 carbon chain fatty acids with a preponderance of 16 carbon and saturated 18 carbon chain fatty acids. The net synthesis and catabolism of lipids were also investigated. Human lung, brain, placenta and liver slices obtained at different periods of gestation were incubated in 95% oxygen-5% CO_2 in the presence of ^{14}C-labeled palmitic acid (PA) (15mc/mM) dissolved in an albumin-phosphate medium, pH 7.35. All tissue incorporated PA into lipids as a function of time of incubation or temperature and of substrate concentration. When tissues were incubated in nitrogen and at 0 C lipid synthesis was dramatically curtailed and the PA radioactivity measured in slices was identical to the zero time control. Fatty acid catabolism was shown by the production of labeled carbon dioxide ($^{14}CO_2$) from PA during incubation. When cell free fractions of lung, brain, liver and placenta of the human fetus were incubated in the presence of 1-^{14}C-acetate in the appropriate medium, lipid synthesis could be demonstrated as early as the first trimester of gestation. The human fetus can, therefore, synthesize lipids and catabolize fatty acids early in gestation, in the absence of cell membranes. The constant concentration and composition of fetal lipids during development shows that the fetus does not accumulate lipids but synthesizes and stores them in the tissue as the organs grow. Since in vitro PA and glucose are the precursors of fetal lipids, and since fatty acids have been shown to cross the placenta in vivo in monkey, rabbit and sheep, and to become incorporated into fetal lipids, lipid metabolism in the human fetus may be regulated by the availability of glucose and fatty acids from the maternal circulation.

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LIPIDS IN HUMAN CERVICAL MUCUS. E. J. SINGH and J. R. SWARTWOUT, University of Chicago, Chicago, Ill. 60637.

The variation of lipid components in human cervical mucus in the menstrual cycle has been determined using combined glass fiber paper chromatography and densitometry. Using florisil column chromatography, the lipids were fractionated into hydrocarbons, cholesteryl esters, cholesterol, triglycerides, diglycerides, monoglycerides, free fatty acids and phospholipids. The purity of the sample was checked by thin layer chromatography. The fatty acid compositions were determined by gas chromatography using polar and nonpolar packings. Cyclic variations in the fatty acid compositions will be discussed. The mucus lipid was also saponified with alcoholic sodium hydroxide and the unsaponifiable portion resolved into hydrocarbons, alcohols and sterol by chromatography over activated alumina. The hydrocarbons are present in the range C_{15} to C_{33}. Analysis of alcohols by gas chromatography of the acetates revealed 45 peaks of range, C_8–C_{32}. Prostaglandins were also isolated and separated by glass fiber paper chromatography. The primary prostaglandins found were $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_1 and PGE_2. It was noted $PGF_{2\alpha}$ is the major component.

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LIPID METABOLISM IN SPERMATOZOA. CHARLES TERNER, Boston University, Boston, Mass. 02215.

Phospholipids are major components of the lipids of spermatozoa and have long been believed to be their substrate for endogenous respiration. The presence in ram spermatozoa of choline plasmalogen and the oxidation of its fatty acid was reported by Hartree and Mann. Our demonstration of the capacity for biosynthesis of glycerides and phosphatides of isolated spermatozoa of mammals including man, and of fish spermatozoa reaffirmed the important role of lipids in the metabolism of the male gamete. Using ^{14}C-labeled glucose, glycerol or glucosamine as precursors of the glycerol portion, and acetate or pyruvate as precursors of fatty acids, diglycerides and triglycerides were found to be the lipids of highest specific activity. Differences in the lipid metabolism of spermatozoa of various animal species are seen in the patterns of incorporation of labeled precursors into glycerides and phosphatides. Current studies include changes in lipid metabolism during the development of the male gamete and its maturation in the epididymis. These studies were facilitated by a rapid and simple technique for the removal from lipid extracts of water-soluble radioactive contaminants.

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LIPID METABOLISM IN TESTIS. J. G. CONIGLIO, B. E. EVANS, JR., and R. ZSELTVAY, Vanderbilt University, Nashville, Tenn. 37203.

The testis is an active organ in the synthesis and metabolism of lipids and in the interconversion of fatty acids. Previous studies (Davis and Coniglio, 1966, and Bridges and Coniglio, 1970) established the biosynthesis of 22:5, 24:4 and 24:5

from the labeled precursors, ^{14}C-linoleate and ^{14}C-arachidonate, injected intratesticularly in the rat. Rat testicular tissue is also active in the synthesis and interconversion of fatty acids in vitro. These studies were conducted with intact pieces of rat testicular tissue incubated in a phosphate buffer with 1-^{14}C acetate. Various factors were studied which might affect incorporation of ^{14}C into total fatty acids or into specific fatty acids. Incorporation of ^{14}C into total fatty acids was stimulated greatly by including glucose in the incubation medium. The most marked changes were in incorporation of activity into 16:0 and 20:3 (increases) and in 22:4 (decrease). The latter fatty acid is present in testicular tissue only in small amounts, but it usually has 10% to 15% of the total ^{14}C incorporated in the fatty acids in an in vitro experiment. It probably results from a simple 2-carbon elongation of the relatively large amount of arachidonate present in this organ. The gas phase also influenced the amount of ^{14}C incorporated into total fatty acids. Incorporation decreased in the order, oxygen > air > nitrogen. Enhanced incorporation with oxygen was primarily associated with 16:0, 18:0 and 18:1, and there were relative decreases in the higher polyenes. With both air and oxygen incorporation of ^{14}C activity was somewhat greater at 25 C than at 37 C. The increase was primarily in 16:0. Although incorporation of ^{14}C-acetate was optimal at pH 7.4, the tissue was active throughout the range of pH tested (4.4–8.4). Total incorporation of ^{14}C in cryptorchid testis (compared to control testis in the same animal) was not decreased, and on the basis of organ weight, the amount of ^{14}C incorporated actually was higher than in the control.

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FATTY ACID POLYMORPHS: CORRELATION OF SOME IR, WIDELINE NMR AND X-RAY DIFFRACTION PARAMETERS. A. V. BAILEY, D. MITCHAM and R. A. PITTMAN, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

Polymorphic modifications of a number of long chain fatty acids were prepared by crystallization at selected temperatures from both polar and nonpolar solvents and from the melt. Crystallization temperature and solvent polarity influence on polymorphic modification formation are reported. Crystal long spacing for both stable and metastable polymorphic forms obtained from x-ray diffraction patterns are correlated with wide line NMR parameters. The second moments calculated from the experimental first derivative curves are linearly related to crystal long spacings. Characteristic IR spectra for each polymorphic modification were obtained in the "fingerprint" region of the spectrum, 750–400 cm^{-1}, and the significance of band shifts is discussed.

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NEW GAS PHASE ANALYTICAL METHODS FOR THE STUDY OF STEROIDS. E. C. HORNING, P. G. DEVAUX, N. SAKAUCHI and M. G. HORNING, Baylor College of Medicine, Houston, Texas 77025.

New procedures for the study of complex mixtures of steroids of biological origin have been developed. It has been found advantageous to employ group reagents which lead to relatively large separations for functional group classes. When O-benzyloxime derivatives are used in place of O-methyloximes, keto- and hydroxy-steroids are completely separated with relatively large differences in retention times. A new silylation procedure, involving the use of N-trimethylsilylimidazole at 120–200 C, has also been developed for the silylation of highly hindered hydroxyl groups. These methods have been used in GC and GC-MS studies of infant and adult human urinary steroids with Dexsil and GE-SE-30 columns. Examples will be discussed.

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ESTIMATION OF *CIS/TRANS* ISOMER CONTENT OF EDIBLE VEGETABLE OILS BY RAMAN SPECTROSCOPY. ROBERT J. HORVAT, R. B. Russell Agricultural Research Center, Athens, Ga. 30604, and GLEN F. BAILEY.

Cis/trans isomer composition of unsaturated glycerides in edible vegetable oils is of interest because *cis* to *trans* isomerization occurs during hydrogenation, and the nutritional value of *trans* fatty acids has been questioned. The Raman spectroscopic analysis described gives the total olefin content along with the *cis/trans* composition of oils, whereas the currently used analytical technique requires both an iodine number and a quantitative IR analysis to give the same information. The Raman analytical technique is based on measuring the intensities of Raman bands near 1656 and 1670 cm^{-1} associated with *cis* and *trans* stretching vibrations of the C = C double bonds. A precision of about 1% was obtained with this technique on binary mixtures of pur *cis* and *trans* methyl esters of both C_{18} monoeinic acid and C_{18} dienoic acid, and their corresponding triglycerides. Methyl esters and triglycerides used in these experiments were examined for purity by gas liquid or thin layer chromatography and IR analysis. Natural and partially hydrogenated oils have been analyzed by using adjusted coefficients to correct for shifts of 1–2 cm^{-1} in C = C scattering moving from oleic to linolenic acid.

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CONDUCTOMETRIC STUDIES OF ISOLATED NATURAL PHOSPHATIDYLCHOLINE IN THE ABSENCE AND PRESENCE OF STEROLS AND FAT SOLUBLE VITAMINS. J. T. HOOGEVEEN, V.A. Hospital, Buffalo, N.Y. 14215.

Isolated natural phospholipids (phosphatidylcholine, phosphatidylethanolamine and sphingomyelin), solubilized in a water-tertiary butanol mixture, show temperature depend phase transitions. In this study, we are mainly concerned with phosphatidylcholine (PC) purified from egg yolk. An optically clear solution of phospholipids at a higher temperature (e.g., 60 C) turns into an opalescent phase via a turbid transitional phase at lowering the temperature. Microscopically, the transitional phase consists of spherical phospholipid droplets. This phase changes into an opalescent phase which is optically clear but shows conductometrically an increase of the resistance. From data in previous studies, it is assumed that this phase consists of a three-dimensional submicroscopic structure formed by lamellar phospholipid membranes. PC shows a considerable increase in resistance which depends on the kind and concentration of cations in the solution. The presence of cholesterol increases the resistance up to 500% depending on the cholesterol/PC ratio. Cholesterol oleate and ergosterol show much smaller effects (160% and 20%, respectively). All the studied fat soluble vitamins (A, D_2, E and K) increase the resistance of the lecithin system; generally an increase of 200% is observed at a vitamin/PC ratio of about 0.3. The hydrocarbons decane and tetradecane, frequently used in model membrane studies, increase the resistance up to 300% at hydrocarbon/PC ratios of approximately 0.1. However, at increased ratios, the resistance dropped rapidly to blanc solvent values, indicating a total disturbance of the PC submicroscopic structure.

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TOTAL SERUM LIPID DETERMINATION BY MEANS OF PULSATING NMR. H. PEETERS and G. A. PERSYN, Simon Stevin Institute, Brugge, Belgium.

Abstract not available at press time.

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DEVELOPMENTS IN STATIONARY PHASES FOR GAS LIQUID CHROMATOGRAPHY ANALYSIS. D. M. OTTENSTEIN, W. R. SUPINA, D. A. BARTLEY and NICHOLAS PELICK, Supelco, Inc., Bellefonte, Pa. 16823.

Developments in stationary phases is the theme of this presentation. Gas chromatograms will be shown to illustrate uses for long and short chain compounds. Some of the phases will be characterized with respect to polarity and type and compared to ones which are currently being used. The new McReynolds constants will be discussed in the classification of the phases.

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ELEMENTAL C, H AND N ANALYSIS OF CRUSHED ROCK AND SOIL SAMPLES. F. T. LINDGREN, G. R. STEVENS and L. C. JENSEN, University of California, Berkeley, Calif. 94720.

A sensitive and reproducible method for elemental analysis of rock and soil samples has been developed using a modified Hewlett-Packard 185 CHN analyzer. Samples are combusted in an inert He atmosphere (with and without catalyst) with a carefully controlled combustion time and temperature. Absolute mass calibration is available on samples from 1–600 μg total CHN content. Total CHN mass reproducibility on duplicate organic standards in the range of from 100–400 μg is approximately 0.5%. Finely crushed rock (or dust) samples of from 50–100 mg may be analyzed for most forms of carbon content with a technical reproducibility of approximately ± 2 to ± 20 ppm over the range of 10–1000 ppm total carbon, respectively. Stepwise calcination at 150 C and 550 C allows evaluation of potential organic carbon content. Although carbide carbon is partially recovered, essentially quantitative recovery of graphitic, amorphous, carbonate and all organic carbon may be obtained. Application of this method has been made to terrestrial and lunar material.

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A SIMPLIFIED HALPHEN PROCEDURE FOR CYCLOPROPENE FATTY ACIDS. EUGENE C. COLEMAN and DAVID FIRESTONE, FDA, Department of Health, Education and Welfare, Washington, D.C. 20204.

A simplified Halphen procedure (based on the method of Bailey et al., 1965) is described for the analysis of fats and oils containing cyclopropene fatty acids. A comparison is made between the response of the samples in butanol and in butanol-dimethyl sulfoxide solvents, since the latter increase sample absorbance. The absorbance is further enhanced by using screw cap test tubes which increase cell (tube) path length and employ a more concentrated sample for absorbance measurement.

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CHARACTERISTICS OF CELLS DISSOCIATED FROM MOUSE MAMMARY GLANDS. S. ABRAHAM, Children's Hospital Medical Center, Oakland, Calif. 94615.

Mammary tissue from lactating mice was dissociated by treatment with collegenase which yielded suspensions of adipose cells and parenchymal cells. Pure preparations of parenchymal cells practically devoid of adipose cells, have been obtained by low speed centrifugations in hypertonic sucrose solution. Mammary parenchymal cells rapidly recover from the osmotic effects of passage through the hypertonic sucrose solution. Measurements of the DNA content indicated that the recovery of parenchymal cells with this procedure was about 20% of the total cells in the original intact tissue. Electon-microscopic examination of the parenchymal cells revealed that the presence of glucose in the dissociation medium prevented the more extreme modifications of organelle and cell-surface structure. To provide evidence for the viability of these isolated pure parenchymal cell preparations, some of their more specific metabolic characteristics were studied and compared to those observed in tissue slices. Parenchymal cells took up glucose and consumed oxygen at higher rates than did the mammary gland tissue slices. Glucose was converted by the cells to carbon dioxide, fatty acid, glycerol, lactate and lactose more rapidly than by the tissue slices. As in the case of tissue slices, both the Embden-Meyerhoff and pentose phosphate pathways were operative in the isolated cells. The contribution of each pathway to the conversion of glucose to fatty acids appeared to be similar in both tissue preparations. Protein and lipid synthesis from leucine and lipid synthesis from acetate were also more extensive in the experiments with the isolated cells. Lipids synthesized from glucose, acetate and leucine by the isolated parenchymal cells were predominantly triglycerides containing the medium chain length fatty acid characteristically produced by the intact gland and found in mouse milk. The specific activities of a number of enzymes involved in lipogenesis from glucose were found to be at least as high in the isolated cells as in the tissue slices. Although there was some leakage of certain enzymes from the cell (pyruvate kinase), and not of others (glucose-6-phosphate dehydrogenase) upon incubation in a medium, similar results were obtained in experiments with slices. There did not appear to be a preferential loss of enzymes from the cells. Thus, since the isolated mammary gland parenchymal cells obtained from lactating mice retain their ability to perform most of the important functions of the intact tissue, they provide an additional tool for the study of mammary gland metabolism and function.

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LIPID METABOLISM IN BRAIN CELLS UNDER CULTURE CONDITIONS. J. H. MENKES, UCLA Center for the Health Sciences, Los Angeles, Calif. 90052.

Tissue explants from frontal lobes of rat brain were used for the study of cerebral fatty acid metabolism. After tissues had been maintained for two days in serum supplemented medium, a lipid-free medium was substituted and metabolic studies were carried out. Under these conditions explants continued to take up lipid precursors for at least 48 hr, as judged by incorporation of D,L-mevalonic acid 2-^{14}C into cellular lipids. 1-^{14}C-stearic acid and 1-^{14}C-palmitic acid were bound to cells as the free fatty acids, or incorporated into neutral lipids (particularly triglycerides), glycolipids and phospholipids. In the galactolipid fraction, cerebrosides were the principal radioactive lipids. Choline, ethanolamine, inositol and serine phosphoglycerides were the principal radioactive phospholipids. Fatty acids were incorporated into cellular lipids either unchanged or after desaturation, chain elongation, or both. Maximum incorporation of stearate occurred in tissues derived from 3-day-old animals. With increasing age the uptake of fatty acid dropped sharply. When the labeling of lipids as a function of time was followed in 3-day-old animals, triglycerides and choline phosphoglycerides were the first fractions to take up stearate. Labeling of cerebrosides occurred slowly, only becoming evident after 24 hr. These studies exemplify the usefulness of tissue explants for prolonged metabolic studies in normal and pathological specimens of brain.

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THE USE OF TISSUE CULTURE TECHNIQUES IN THE STUDY OF THE LIPID STORAGE DISORDERS. H. R. SLOANE, National Institutes of Health, Bethesda, Md. 20014.

The technique of tissue culture was first successfully applied to the study of galactosemia in 1962 and since that time has been widely applied to the study of many metabolic disorders. All of the enzymes associated with the lipid storage diseases are present in tissue culture cells derived from normal individuals. The eight major lipid storage disorders can be diagnosed by measuring the activity of these enzymes in cultured fibroblasts. Some of the properties of the mutant enzymes in Niemann-Pick disease have been evaluated with fibroblast preparations. New techniques permit the establishment of perpetual lymphocyte cultures from small samples of venous blood and the study of these lines opens new avenues for the study of metabolic disorders. A simple histochemical test for G_{M1} gangliosidosis has been developed and applied to both cultured fibroblasts and cultured white cells. Both types of cells are excellent sources of radioactively labeled sphingolipids that may be employed in enzymatic studies of the lipid storage disorders. Culture of the fetal cells desquamated into the amniotic fluid permit a preview of the fetus's potential enzyme profile and thereby enable early intrauterine diagnosis of metabolic disorders. Several of the lipid storage disorders have already been diagnosed in utero, and the tragedy associated with the birth of such a handicapped child has thereby been avoided.

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THE USE OF CULTURED FETAL CELLS FOR THE ANTENATAL DIAGNOSIS OF LIPID STORAGE DISEASES. R. O. BRADY, National Institutes of Health, Bethesda, Md. 20014, B. W. UHLENDORF, C. B. JACOBSON and C. J. EPSTEIN.

One of the most important contributions derived from studies of lipid metabolism in cultured cells is the observation that the metabolic alterations which occur in inheritable human diseases are accurately reflected in fetal cells in culture. Thus, assays for the activity of the enzymes involved in Gaucher's disease (glucocerebrosidase), Niemann-Pick disease (sphingomyelinase) and Fabry's disease (ceramidetrihexosidase) permit the prenatal detection of fetuses afflicted with these conditions. We have shown, through the use of radiocarbon-labeled natural sphingolipids and chromogenic artificial substrates, that the enzymes involved in these conditions are present in good activity in extracts of normal human cells obtained by amniocentesis and subsequently grown in tissue culture. With these techniques, we have recently monitored pregnancies at risk for Gaucher's disease, Niemann-Pick disease and Fabry's disease. The results of these determinations will be presented at this symposium.

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LIPOPROTEIN ABNORMALITIES IN FAMILIAL LCAT DEFICIENCY. K. R. NORUM, JOHN GLOMSET, University of Washington, Seattle, Wash. 98101, A. V. NICHOLS and TRUDY FORTE.

The plasma lipoproteins of patients with familial lecithin-cholesterol acyltransferase (LCAT) deficiency provide striking evidence of the physiological importance of the LCAT reaction in Man. The composition and physical properties of these lipoproteins are highly abnormal, and these abnormalities are probably caused by the enzyme defect since the lipoproteins are altered toward normal upon reacting with partially purified LCAT from normal plasma. The very low density lipoproteins (VLDL) contain less cholesteryl ester than normal and migrate as beta rather than prebeta lipoproteins on electrophoresis, but increase both in cholesteryl ester content and in mobility upon incubation with LCAT in the presence of normal high density lipoproteins (HDL). The low density lipoproteins (LDL) are comprised of at least two molecular species. One is of large molecular weight and contains 7–10 times as much unesterified cholesterol (C), phosphatidylcholine (PC) and triglyceride (TG) per mg protein as normal LDL; the second is of smaller molecular weight and contains 1.5–3 times as much C and PC and 13 times as much TG per mg protein as normal LDL. Both contain much less cholesteryl ester than normal, and the compositions of both are altered by incubation with LCAT and HDL. The HDL also are comprised of at least two molecular species. One, of large molecular weight, contains about 12 times as much C and 5 times as much PC per mg protein as normal HDL; a second, of smaller molecular weight, contains only slightly more C and PC than normal HDL. Again, both contain less cholesteryl ester than normal and the compositions of both are altered by reaction with LCAT.

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STRUCTURE OF LIPOPROTEINS FROM LCAT DEFICIENT PLASMA. TRUDY FORTE, University of California, Berkeley, Calif. 94704.

The chemical and physical properties of lipoproteins from lecithin:cholesterol acyltransferase (LCAT) deficient plasma are highly abnormal; this is especially true for the low density (LDL) and high density (HDL) lipoproteins. Their morphology, as seen with the electron microscope after negative staining, is also abnormal. LDL are heterogeneous in size and show the following two distinct populations of particles: small, approximately spherical particles 210–250 A in diameter which are similar to normal LDL, and large, flattened structures with diameters between 900–1200 A. The HDL also show heterogeneity; the majority of particles are disc-shaped structures 150–200 A in diameter although there is a small percentage of particles 45–60 A in size. The disc-shaped structures appear to be composed of a rosette of small (50 A) globular units. During the process of drying in the negative stain, the disc-like structures aggregate in stacks which have a periodicity of 50–55 A. Such an arrangement contrasts with that of normal HDL, which are 70–100 A in diameter, and aggregate in monolayers with hexagonal packing of the particles. The disc-shaped HDL structures are presumably a result of the near absence of nonpolar lipids and the presence of a high proportion of polar lipids, namely, phospholipid and cholesterol. In vitro studies on sonicated mixtures of HDL peptides plus phospholipids or phospholipids and cholesterol produced stacked discs with 50–55 A periodicity which are very similar to the HDL structures isolated from LCAT deficient plasma.

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LCAT REACTIVITY AND SUBSTRATE INTERACTIONS. A. V. NICHOLS, WALTER HO and TRUDY FORTE, University of California, Berkeley, Calif. 94704.

During incubation of human serum in vitro, the substrates of the enzyme lecithin:cholesterol acyltransferase are the unesterified cholesterol and lecithin of the serum lipoproteins. Sonicated dispersions of defined mixtures of labeled unesterified cholesterol with lecithin can serve as substrates for radio assay of acyltransferase activity. Net esterification yields (24 hr incubation) and initial reaction rates decrease with increasing molar proportions of unesterified cholesterol in the sonicated substrate. Negligible acyltransferase activity is observed when lecithin in sonicated substrates is replaced by phosphatidyl ethanolamine, phosphatidyl serine or sphingomyelin. Other factors (sulfhydryl reagents, lipoproteins, lipoprotein lipids and/or peptides) influencing the reactivity of sonicated substrates will be discussed. Factors affecting distribution of apolar product cholesteryl esters among substrate particles and/or serum lipoproteins were evaluated. The effects of accumulation of cholesteryl esters on physical properties of model substrates were determined; marked structural changes were observed by electron microscopy in peptide-containing substrates following enzymatic or sonic incorporation of cholesteryl esters. Radio-assay of acyltransferase activity in the ultracentrifugal protein fraction ($d > 1.21$ g/ml) of 10 human subjects was performed. During incubation with the ultracentrifugal protein fraction, sonicated dispersions of lecithin form complexes containing acyltransferase activity. These complexes can be isolated by ultracentrifugal flotation in sucrose (d 1.065 g/ml). Complex formation is not affected by inhibition of the enzyme's activity with hydroxymercuribenzoate. Enzyme activity is partially inhibited by increasing concentrations of lecithin. The lecithin-acyltransferase complexes can be dissociated ultracentrifugally after treatment with sodium taurocholate and high concentrations of KBr. By this method the enzyme was purified approximately 500-fold.

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ON THE NATURE OF A LIPOPROTEIN (LP-X) CHARACTERIZING THE BILIARY OBSTRUCTION AND ITS POSSIBLE RELATIONSHIP TO LCAT. P. ALAUPOVIC, H. MAGNANI and D. SEIDEL, Oklahoma Medical Research Foundation, Oklahoma City, Okla. 73104.

Plasma lipids in subjects with biliary obstruction are characterized by increased concentrations of unesterified cholesterol and phospholipids and the lipoprotein spectrum by an increased content of low density lipoproteins. By developing a procedure for the separation of lipoprotein families (1969) we confirmed Switzer's original observation (1967) that the characteristic elevation of unesterified cholesterol and phospholipid concentrations is due to the presence of an abnormal low density lipoprotein, designated as LP-X. Whereas the LP-B family was characterized by an almost normal lipid composition and apolipoprotein B as the protein moiety, the LP-X contained mainly unesterified cholesterol (20–22%) and phospholipid (65–66%) as lipid components and a protein moiety (5–6%) consisting of a mixture of approximately 40% albumin and 60% the specific apolipoprotein, ApoX. The antigenic site of albumin can be revealed only by partial or total delipidization of LP-X. Apolipoprotein X was characterized by serine and threonine as the major N-terminal and alanine, valine and glutamic acid as the major C-terminal amino acids. Results of electrophoretic and immunological studies indicated that ApoX represents a complex protein consisting of at least three distinct polypeptide components. Direct comparison of chemical and immunological properties indicated that ApoX is similar, if not identical, to apolipoprotein C isolated from very low density lipoproteins of normal or hyperlipemic subjects. The possible relationship of increased plasma unesterified cholesterol and LP-X with decreased activity of LCAT in patients with obstructive jaundice will be discussed.

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EFFECTS OF LIPID PEROXIDE ON CHOLESTEROL ESTERIFICATION. H. S. MICKEL, E. L. FOULDS and D. A. CLARK, School of Aerospace Medicine, Brooks AFB, Tex. 78235.

Previous studies of young men breathing 100% oxygen at reduced prssure (5 psi) revealed a 40% drop in the serum lecithin:cholesterol acyltransferase actviity during the fourth week of exposure to oxygen. To explore possible mechanisms by which increased oxygen pressure might affect the activity of this enzyme, a series of in vitro incubations was conducted with serum plus mercaptoethanol (ME), *p*-chloromercuribenzoate (PCMB), hydrogen peroxide, or various peroxidized lipids (PxL) (peroxidized lecithin, cholesteryl linoleate or trilinolein). PCMB inactivated the enzyme but ME restored the activity and protected the enzyme if added before PCMB. Hydrogen peroxide also inactivated the enzyme but ME partially restored the activity. PxL reduced the initial enzyme activity 20–50% over a 24 hr period. Reversal of this inhibition by ME was complete if exposure of the enzyme to PxL was less than 1 hr. After 24 hr exposure of the enzyme to PxL, ME restored enzyme activity only partially (65% of control value). Reversible inhibition of lecithin:cholesterol acyltransferase was attributed to reversible oxidation of the —SH groups into —SS— bridges. Irreversible inhibition may represent the reaction of lipid peroxide with the substrate or, more likely,

the enzyme protein. Inhibition of acyltransferase by one of these mechanisms is evidently a pathway by which hyperoxia may affect a facet of cholesterol metabolism.

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INFLUENCE OF APOPROTEINS ON LIPOPROTEIN STRUCTURE AND LIPID TRANSPORT. A. M. GOTTO and S. E. LUX, National Heart and Lung Institute, Bethesda, Md. 20014.

Recently, considerable research has been stimulated by the discovery of apoprotein heterogeneity in the plasma lipoproteins. The six principal proteins (R-ser, R-val, R-glu, R-ala, R-thr and R-gln) are present in different proportions in very low density (VLDL), low density (LDL) and high density (HDL) lipoproteins. R-ser, the protein moiety of LDL, is also found in VLDL (40–50%). R-val, R-glu and R-ala are major components of VLDL (50–60%) and are found as minor components (5–15%) of HDL. The major HDL apoproteins are R-thr (65–70%) and R-gln (20–25%). The interactions between apoproteins and lipids have been studied directly in dehydration-rehydration experiments and with circular dichroism (CD) and have been investigated indirectly through the use of two human genetic disorders, Tangier Disease and Abetalipoproteinemia. The dehydration-rehydration experiments suggest that R-gln has a greater lipid affinity than R-thr. By CD criteria these two proteins have different helical contents both before and after reconstitution with lipids. Both phospholipids and neutral lipids are required for complete reorganization of the secondary and tertiary structure of HDL. In Tangier Disease, where normal HDL is absent, and a beta-lipoproteinemia, where LDL, VLDL and chylomicrons are all absent, the distribution of apoproteins gives valuable insight into their normal roles in the physiological transport of lipid.

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DIFFERENTIATION OF LIPIDS IN PLASMA LIPOPROTEINS. V. BLATON, Simon Stevin Instituut voor Wetenschappelijk Onderzoek, Jerusalemstraat 84, B-8000 Brugge, Belgium.

As the plasma lipids are protein bound their accurate picture can only be obtained through a fingerprint of the individual lipids isolated from individual lipoproteins. A group of methods has been developed and combined to establish a fingerprint of lipids, lipoproteins and fatty acids. Lipids from lipoproteins separated by electrochromatography (ECG), ultracentrifugation (UCF) and dextran precipitation are compared. The purity of isolated lipids by column chromatography on SiO_2, Al_2O_3, DEAE-cellulose and Sephadex LH-20, and by uni- and bidimensional thin layer chromatography is checked by gas chromatography and infrared spectroscopy. Plasma lipids have been investigated in detail in normal human subjects, in type II essential familial hyperlipemia (EFH), and in experimental atherosclerosis with chimpanzees and baboons given an atherogenic diet. The integrated method of lipid analysis in plasma lipoproteins was able to demonstrate the entity of high and low density lipoproteins and indicates that the pathological effects of the atherogenic diet are due not only to an increased plasma level of beta lipoproteins but also to a change in composition which decreases their stability. The fatty acid analysis from control and atherogenic animals can be correlated with those from human subjects, where similar trends are observed in comparisons between control patients and patients with type II disease.

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COMPARATIVE STUDY OF LIPOPROTEINS IN PRIMATES. H. PEETERS, Simon Stevin Instituut voor Wetenschappelijk Onderzoek, Jerusalemstraat 34, B-8000 Brugge, Belgium.

From extensive studies on cebus monkeys, baboons and chimpanzees under an atherogenic diet it appears that lipoprotein levels and the lipid composition of these lipoproteins triggering the reaction of the arterial wall, or concomitant with the disease, are variable. The morphological modifications are reinterpreted in function of more complete biochemical data on lipoprotein distribution in the plasma of primates. A comparative study of lipoproteins from normal primates, separated and compared by electrochromatography, paper and agarose electrophoresis and ultracentrifugation and interpreted according to Fredrickson shows a progressive increase in high to low density lipoproteins according to the species and chromosome differences. All primates have more alpha lipoproteins than man. The plasma lipoproteins of the primate species change in a different way under a same atherogenic diet. Only chimpanzees modify their lipoprotein spectrum in a way similar to human patients. The subunits of the apolipoprotein (apo-HDL and apo-LDL) separated by electrofocusing are correlated to human results. Since the apoprotein carries the lipid load, the molecular and genetic variation of the protein moiety of the lipoprotein is claimed to be responsible for the differences in the response to atherogenic influences.

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LIPID COMPOSITION AND PERMEABILITY OF MEMBRANES. L. L. M. VAN DEENEN, State University of Utrecht, Vondellaan 26, Utrecht, The Netherlands.

The importance of the chemical makeup of phospholipids and the phospholipid-sterol ratio for barrier properties of membranes has been demonstrated for both artificial and natural membranes. The permeability of liposomes for nonelectrolytes was found to increase with increasing unsaturation of the fatty acid constituents. A similar relationship was established for the permeability of intact cells of *Mycoplasma laidlawii* B in which the fatty acid composition was altered by means of the growth medium. In agreement with predictions based on permeability studies of liposomes, the presence of cholesterol in the natural membrane was found to reduce the rate of penetration of nonelectrolytes. Studies on the valinomycin-induced permeation of $^{86}Rb^{+}$ from liposomes demonstrated that the promoting effect of valinomycin is dependent on the degree of unsaturation of the hydrocarbon chains of phospholipids, the presence of sterol and the nature of the polar head groups of phospholipids. The results support the conclusion that the chemical composition of membrane lipids governs the rate of both simple and facilitated diffusion across membranes. Relevant aspects of enzymatic control of phospholipid composition and nonuniformity of membrane structure will be discussed. In Tangier Disease, where normal HDL is absent, and Abetalipoproteinemia, where LDL, VLDL and chylomicrons are all absent, the distribution of apoproteins gives valuable insight into their normal roles in the physiological transport of lipid.

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PROTEIN-LIPID INTERACTION IN THE ERYTHROCYTE MEMBRANE. R. F. A. ZWAAL, University of Utrecht, Vondellaan 26, Utrecht, The Netherlands.

The molecular associations between proteins and lipids in mammalian red cell membranes have been studied by carrying out in vitro recombinations with apoproteins solubilized from erythrocyte ghosts and aqueous lipid aggregates. It appeared that opposite charges are of paramount importance for their initial interaction, since no recombination occurs at physiological pH, probably due to repulsion of both negative net charges of proteins and lipids. At pH 4, however, neuraminidase-treated, positively charged apoproteins are found to recombine with negatively charged lipids up to a phospholipid-protein ratio, comparable to the original membrane. Proton magnetic resonance and electron spin label studies strongly suggest that subsequently apolar interactions between the proteins and the fatty acid moieties of the lipids are formed, which is in agreement with the observation that once the complex has been associated, addition of strong electrolytes or change in pH is ineffective in disrupting the complex. In addition, the reconstituted lipoproteins are found to display a similar heterogeneity in lipid-binding in terms of extractability by organic solvents of increasing polarity, as detected for red cell ghosts. Comparative electron microscopic studies demonstrated that the reconstituted lipoproteins partly show vesicular structures with similar trilaminar images as observed in erythrocyte membranes. It is concluded that these in vitro recombinations first involve electrostatic attractions which are followed by hydrophobic associations.

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LIPID-PROTEIN ASSOCIATIONS IN MEMBRANES: EVIDENCE FOR HYDROPHOBIC AND POLAR INTERACTIONS IN MITOCHONDRIAL STRUCTURE. G. LENAZ, A. M. SECHI, G. PARENTI-CASTELLI and J. CABO SOLER, University of Bologna, Bologna, Italy.

An aliquot of the mitochondrial proteins is not easily detachable from the membrane; such insoluble proteins appear to be linked to phospholipids mainly by hydrophobic interactions. Succinoxidase and NADH-oxidase activities are restored by different types of phospholipids in lipid-depleted mitochondria, but certain lipids, such as those from myelin, which contain a large proportion of saturated and long chain fatty acid residues, are much less effective; unsaturation and/or an optimum chain length of the fatty acid residues in the lipids appear to be required for hydrophobic interaction with respiratory chain proteins. Another aliquot of mitochondrial proteins appears extractable by certain treatments (diluted acid, salts) or can be digested with proteolytic enzymes. Studies on submitochondrial particles demonstrate that among these proteins are constituents of the ATPase complex. Such proteins are linked to the membrane by weaker bonds which appear to be determined, at least in part, by polar interactions with phospholipids. Treatments inducing detachment of the easily soluble proteins increase the extractability with ether or pentane of phospholipids from the membrane. The possibility of a role of Mg^{++} in these interactions is discussed. Polar bonds between phospholipid and protein are also suggested by the possible presence of proteolipids soluble in organic solvents.

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INTRACELLULAR TRANSPORT OF PHOSPHOLIPIDS IN LIVER. K. W. A. WIRTZ, University of Utrecht, Vondellaan 26, Utrecht, The Netherlands.

Phospholipids exchange between liver mitochondria and microsomes in vitro. Both inner and outer mitochondrial membranes are involved. Phospholipid exchange between mitochondria and microsomes is stimulated by a protein fraction of the 105.000 × g supernatant, denoted as phospholipid exchange protein. Phosphatidylcholine exchanges more rapidly than phosphatidylethanolamine. The transfer of phospholipids between outer and inner mitochondrial membrane is not stimulated by a soluble mitochondrial protein. Within the mitochondrion, phosphatidylcholine and phosphatidylethanolamine exchange equally well. Purification of phospholipid exchange protein from beef heart has resulted in a highly active fraction, which stimulates specifically the exchange of phosphatidylcholine and phosphatidylinositol. Phospholipid exchange protein from rat heart, liver, kidney and brain stimulate the exchange of the various classes of phospholipids between liver mitochondria and microsomes to different extents. All the available evidence suggests that specific proteins participate in the exchange of each phospholipid.

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STUDIES ON THE LOCALIZATION OF CERTAIN LIPIDS IN SURFACE MEMBRANES OF *TETRAHYMENA PYRIFORMIS*. YOSHINORI NOZAWA, RAYMOND BAMBERY, ANNITA WEIDENBACH and G. A. THOMPSON, JR., The University of Texas, Austin, Texas 78712.

We have recently reported an enrichment of phosphonolipids in surface membranes of *Tetrahymena*. These lipids account for nearly 70% of the lipid phosphorus of ciliary membranes. This is more than twice the concentration found in the cytoplasmic membranes of the cell. We have also found other differences in the lipid composition. The triterpenoid tetrahymanol, which replaces sterols in this organism, is more than ten times more concentrated with respect to phospholipids in the ciliary membranes than in internal membranes. The glyceryl ether content of surface membrane lipids is also elevated. Since these lipids are made in the cell interior, principally by microsomal enzymes, the question arises as to what mechanism allows their localization in outer regions of the cell. We have data from isotope studies indicating that the assortment of lipids reaching the ciliary membranes during membrane growth may be virtually identical to that found internally. Some mechanism seems to remove from the membranes certain newly inserted lipid species, leaving others. Incubation of isolated membrane fractions reveals that some of these, including the cilia and the other surface membrane-containing fraction, have lipolytic enzymes capable of degrading phospholipids. The lipids which we have found to accumulate in surface membranes are largely spared by the degradative enzymes. We shall present a hypothesis which explains how the enrichment of enzymatically stable lipids might occur at the cell surface and how this could benefit the cell.

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STRUCTURAL ASPECTS OF ERYTHROCYTE MEMBRANE PROTEINS. P. ZAHLER and E. WEHRLI, Theodor Kocher-Institute, Freiestrasse 1, Berne, Switzerland.

It could be shown that large parts of rbc membrane proteins are in α-helical conformation. In 2-chloroethanol the lipids are readily separated from the proteins without affecting largely the protein formation. For the isolation of single membrane proteins, Sephadex G-200 filtration in 8 M urea and 0.5% SDS was used. The isolated coiled proteins by transferring to 2-chloroethanol can be changed to the initial helical conformation, as shown by circular dichroism measurements. These apoprotein fractions again bind lipids and form membranous aggregates as shown by electron microscopy. The chemical and structural aspects of these reconstituted membranes will be discussed.

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THE INTERACTIONS OF PROTEINS ISOLATED FROM ERYTHROCYTE MEMBRANES. A. H. MADDY, University of Edinburgh, Department of Zoology, West Mains Road, Edinburgh EH9 3JT Scotland.

Proteins may be isolated from membranes either by a series of extractions with a sequence of reagents of increasing severity or by a one-step procedure aimed at releasing all the protein into solution by exposure of the membrane to just one solvent. A butanol procedure has the merit of producing virtually all the protein of erythrocyte ghosts in an aqueous solution in a relatively undamaged state, but unfortunately this protein solution is difficult to fractionate. However, if the butanol procedure is carried out at low ionic strength in the presence of EDTA the resulting protein solution can be readily fractionated. Using acrylamide gel electrophoresis and analytical centrifugation we have demonstrated in this solution (a) a fraction identical with that part of the membrane's protein that may be extracted from the ghost by EDTA solution alone, et al., (b) a fraction containing most and probably all of the membrane's sialoprotein and surface antigens, and (c) a component with a pronounced tendency to aggregate into an intractable complex. Three sets of interactions have been detected as factors responsible for aggregation of the isolated membrane proteins: a hydrophobic force, identified by the effects of increasing the ionic strength of the solution, a divalent ion bridge effect reversed by EDTA or low pH, and an electrostatic interaction between sialoproteins and other protein of opposite charge. Other interactions not destroyed by SDS or guanidine hydrochloride have been observed but their physical basis is unknown. The influence of these forces on the behavior of ox erythrocyte membrane proteins after an EDTA/butanol isolation will be considered. The relative effects of the interactions will differ in different methods of protein isolation, but whatever method is employed their existence must be recognized and accounted for. They are also of relevance in a consideration of the interactions of the proteins with lipids.

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STUDIES ON THE ROLE OF STEROLS IN YEAST CELL METABOLISM. L. W. PARKS, P. R. STARR and E. D. THOMPSON, Oregon State University, Corvallis, Ore. 97331.

Although the sterols have been one of the most intensively investigated groups of biological components, an inordinately small amount of information is available concerning their role in the metabolism of the cell. Our experiments have been aimed toward understanding those factors, cultural, enzymatic and genetic, which affect the rate of synthesis and final cellular yield of sterols in yeast. The amount of sterol varies in yeast depending upon the strain and cultural conditions. From 2% to 5% of the dry weight of the organism may be ergosterol. A relationship between ergosterol production and aerobic metabolism is well-documented. Under anaerobic conditions, squalene is accumulated which upon aeration is rapidly converted to ergosterol by the cells. Ergosterol occurs both as free sterol and esterified to fatty acids. Differences in extractability have been interpreted to indicate an acid-labile and base-labile form of the sterols in the cells. The proportion of sterol in these forms shifts during respiratory adaption. When yeast cytochrome oxidase is purified, sterol remains associated with the enzymatic activity. The close similarity in the kinetics of appearance of ergosterol and cytochrome oxidase activity during respiratory adaption is being studied further. A mutant of *S. cerevisiase* has been obtained which synthesizes only very small amounts of ergosterol. A comparison of the properties of this organism with those of a wild type clone will be reported.

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EFFECTS OF STEROLS ON GROWTH AND DEVELOPMENT IN *PHYTOPHTHORA CACTORUM*. C. G. ELLIOTT, University of Glasgow, Glasgow, W.2., Scotland.

Phytophthora cactorum can grow vegetatively on medium without sterols, although it is unable to synthesize them. Addition of sterol to the medium is required for sexual reproduction to occur; vegetative growth is also increased. These effects can be separated by the use of sterols of different molecular structure and by adding sterol to partially grown cultures.

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STEROID NUTRITION AND METABOLISM IN *PYTHIUM* AND *PHYTOPHTHORA*. J. W. HENDRIX, University of Kentucky, Lexington, Ky. 40506.

The *Pythiaceae* appear at this point to have in common several unique characteristics: phytopathogenicity (assumed for all, demonstrated from many), inability to synthesize sterols, ability to grow vegetatively in the absence of sterols, a sterol requirement for sexual or motile spore reproduction, a Ca^{2+} requirement (which may be unusual for fungi), tolerance for polyene antibiotics, tolerance for gallic acid (many but not all species) and sensitivity to chloramphenicol, the inhibitor of prokaryotic organisms (shown for only one isolate). Thus these fungi share certain characteristics with prokaryotic organisms and with eukaryotic organisms. Pythiaceous fungi take up cholesterol much more efficiently than other fungi and metabolize cholesterol to at least two compounds, one an unidentified polar compound. Both the Pythiaceae and other fungi synthesize esters from cholesterol. Cholesterol uptake and production of the polar metabolite are correlated with the growth phase in *Pythium periplocum*, but production of sterol esters occurs slowly during growth and reaches a peak during autolysis of mycelium or maturation of oospores.

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STEROLS IN RELATION TO NONSENSITIVITY OF PYTHIACEAE FUNGI TO POLYENE ANTIBIOTICS. P. H. TSAO, University of California, Riverside, Calif. 92502.

The antifungal polyene antibiotics (e.g., filipin, nystatin and pimaricin) inhibit growth of fungi and other sensitive organisms by altering the selective permeability of the cell membrane, resulting in leakage of essential intracellular constituents. The cell membrane is the site of action of the polyenes, and the antibiotic-binding component is believed to be the membrane sterols. Microorganisms known to contain no sterols, such as most bacteria and blue-green algae, are nonsensitive to the polyenes. Interestingly, the growth of Pythiaceae fungi (*Phytophthora* and *Pythium*) is also not inhibited by the polyenes, some of which have been used advantageously as selective ingredients in isolation media for these soil pathogens. Many Pythiaceae fungi neither synthesize sterols nor require them for vegetative growth; their nonsensitivity to the polyenes has been attributed to a lack, or less amount, of sterols in their mycelia. Some nonsensitive Pythiaceae become sensitive to the polyenes after being grown in media containing sterols, presumably by incorporating exogenous sterols into the cell membrane. Mycelia of Pythiaceae fungi are less sensitive to the polyenes than nonmycelial propagules (e.g., chlamydospores, sporangia and zoospores). Spore germination is inhibited, and nonencysted naked zoospores are lysed, by antibiotic concentrations which are noninhibitory to mycelial growth. Since sterols are required for the formation of reproductive structures of Pythiaceae and for zoospore cleavage in the sporangia, the differential sensitivity of spores and mycelia of these fungi to the polyenes might be attributable to a higher amount of sterols in the spore membrane. Sterols may also play a role in the resistance of these spore propagules to adverse environmental conditions, since the spore populations that are resistant to the polyenes (thus presumably containing less or no sterols) are those sensitive to cold, desiccation and aging, and those sensitive to the antibiotics are resistant to adverse conditions.

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ROLE OF STEROIDS IN DIFFERENTIATION OF *PHYMATOTRICHUM OMNIVORUM*. H. E. BLOSS, University of Arizona, Tucson, Ariz. 85721.

The soil-borne fungus, *Phymatotrichum omnivorum*, parasitizes roots of more than 2000 species of dicotyledonous plants in the southwestern and south central United States and is disseminated throughout northern Mexico. The fungus produces heavy, plaited strands and sclerotia when supplied with soluble starch and nitrogen. Asexual conidia are produced under light on an agar medium amended with 28-carbon sterols, ergosterol, lumisterol-3, β-sitosterol or β-stigmasterol. The formation of conidiophores and conidia requires a sterol and cool white or blue fluorescent light, 1804–2788 $\mu W/cm^2$. Production of viable conidia is attended by the formation of a yellow-tan pigment. The fungus forms, under light, clamp connections and basidia bearing sexual basidiospores when 1 ppm vitamin D_3 is supplied. The role of calcium ions and the intact steroid nucleus in asexual reproduction and the role of substituents of carbons 24 and 25 in the mechanism of differentiation toward sexuality are discussed.