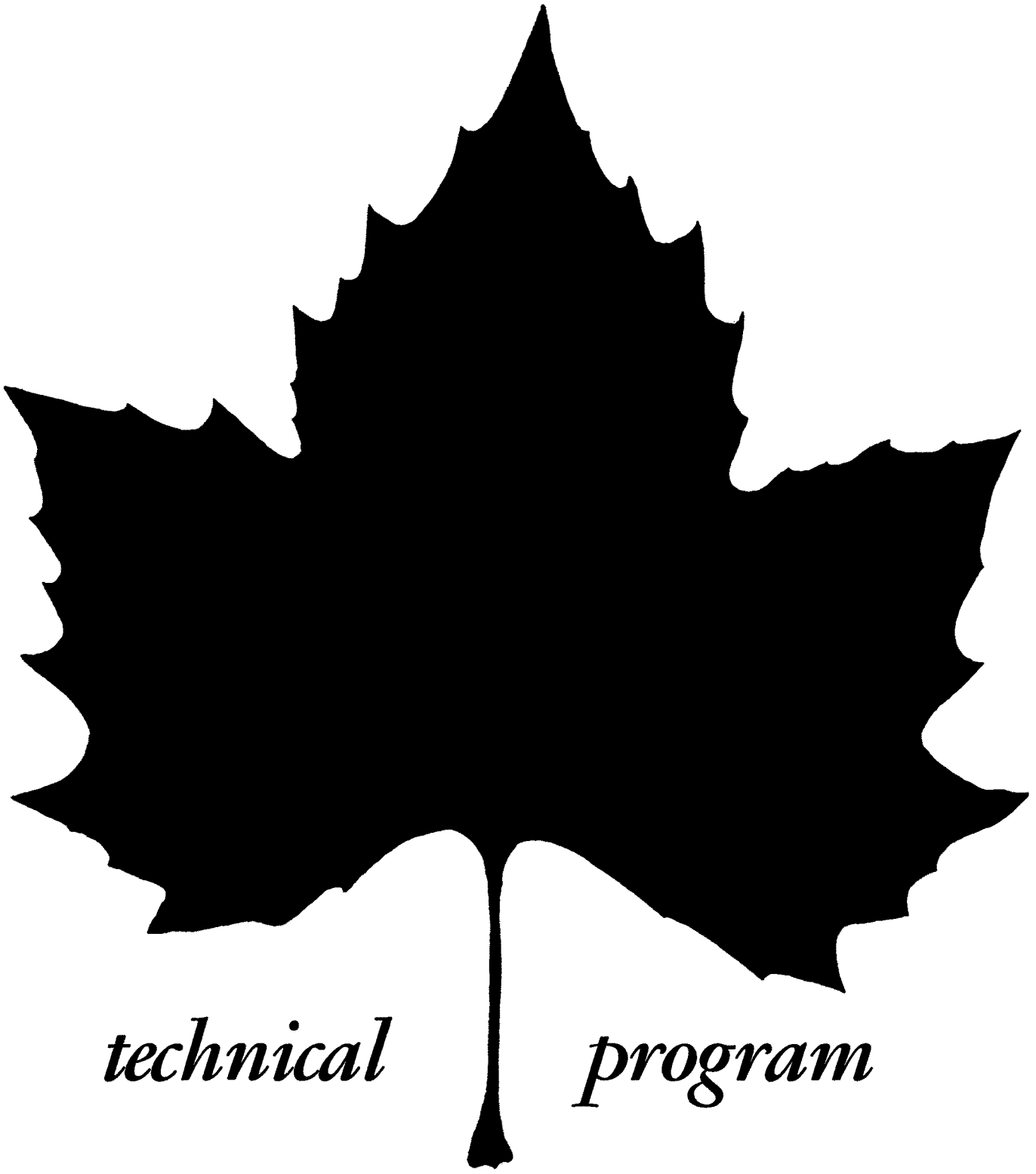

*technical program*

# *AOCS 46th annual fall meeting*

*Ottawa, Canada, Sept. 24-28, 1972*

## MONDAY MORNING—SEPTEMBER 25

**10:30 A.M.—Adam Room**

## SESSION A—GENERAL: BIOCHEMISTRY

Chairman—**F.D. Sauer,** Animal Research Institute, Dept. of Agriculture, Ottawa, Ont., Canada

**10:30** **1. EMBRYONIC TISSUE LIPIDS: THE STATIC AND DYNAMIC STATES OF GLYCERIDES IN VARIOUS TISSUES DURING DEVELOPMENT**
Randall Wood and Daniel H. Winship, University of Missouri School of Medicine

**10:50** **2. LIPIDS OF HUMAN TESTICULAR TISSUE**
John G. Coniglio and Robert K. Rhamy, Vanderbilt University

**11:10** **3. UTERINE LIPID RESPONSE OF OVARIECTOMIZED RATS TO HORMONAL ADMINISTRATION**
P.T. Russell, Bruce C. Moulton and William J. Miller, University of Cincinnati Medical Center

**11:30** **4. IN VITRO HEPATIC CHOLESTEROGENESIS IN RATS FED CORN OIL AND FAT-FREE DIETS AND ITS INHIBITION BY EXOGENOUS LINOLEATE**
P.O. Egwim and K.W. Kummerow, Burnsides Research Lab., University of Illinois

## MONDAY MORNING—SEPTEMBER 25

**10:30 A.M.—Convention Hall**

## SESSION B—MARINE AND GENERAL

Chairman—**R.F. Addison,** Fisheries Research Board of Canada, Halifax, N.S., Canada

**10:30** **5. EFFECT OF TERTIARY BUTYLHYDROQUINONE AND OTHER ANTIOXIDANTS ON OXIDATIVE STABILITY OF MARINE OILS. PART I: WHALE OIL**
M.H. Chahine and R.F. MacNeill, Nova Scotia Research Foundation

**10:50** **6. HONORED STUDENT PRESENTATION**
**DOCOSAHEXAENOATE BIOSYNTHESIS BY A HETERTROPHIC, MARINE DINOFLAGELLATE**
David H. Beach, Upstate Medical Center

**11:10** **7. THE PRACTICAL ASPECTS OF FATS AND OILS IN FRYING**
B.L. Thomas and K.H. Goertemiller, B.L. Thomas Associates

**11:30** **8. EFFECTS OF FEEDING TRIUNDECANOIN TO COWS AND RATS**
Joel Bitman, R.W. Miller, T.R. Wrenn and L.P. Dryden, USDA

## MONDAY MORNING—SEPTEMBER 25

**10:30 A.M.—Banquet Room**

## SESSION C—SYMPOSIUM: PROCESSING OF EDIBLE OILS

Chairman—**Bart Teasdale,** Canada Packers Ltd., Toronto, Ont., Canada

**10:30** **9. TRENDS IN PROCESSING AND MANUFACTURING IN EDIBLE OIL REFINERIES**
Ben W. Minshew, Archer Daniels Midland Co.

**11:00** **10. CLAY-HEAT REFINING OF EDIBLE OILS**
T.K. Mag, Canada Packers Ltd.

**11:30** **11. APPLICATION OF PARTIAL HYDROGENATION THEORY TO THE DESIGN OF COMMERCIAL REACTORS FOR HYDROGENATING TRIGLYCERIDE OILS**
Lyle F. Albright, School of Chemical Engineering, Purdue University

## MONDAY MORNING—SEPTEMBER 25

**10:30 A.M.—Salle Richelieu Room**

## SESSION D—SYMPOSIUM: ANALYSIS OF POLYMERIZED PRODUCTS IN FATTY ACIDS—OILS AND DERIVATIVES

Chairman—**A.E. Waltking,** Corn Products Corp., Union, N.J.

**10:30** **12. THE CHEMICAL ANALYSES OF POLYMERIZATION PRODUCTS IN ABUSED FATS AND OILS**
A.E. Waltking, Wm. Seery and Geo. Bleffert, Corn Products Corp.

**11:00** **13. THE APPLICATION OF GEL PERMEATION CHROMATOGRAPHY TO HEATED FATS**
E.G. Perkins, R. Taubold and A. Hseih, Burnsides Research Lab., University of Illinois

**11:30** **14. THE PHOTO-DIMERIZATION PRODUCTS OF UNSATURATED FATTY ACID METHYL ESTERS**
Osamu Suzuki, Tetsutaro Hashimoto, Government Chemical Industrial Research Institute

## MONDAY MORNING—SEPTEMBER 25

**10:30 A.M.—Renaissance Room**

## SESSION E—SURFACTANTS AND DETERGENTS

Chairman—Name not available at press time.

**10:30** **15. VARIABLES IN THE POWDERING OF FLOOR FINISHES**
M.E. Ginn, E.T. Fronczak and J.H. Junkin, Masury-Columbia Co.

**10:50** **16. AUTOMATED DETERMINATION OF SILICATE AND CARBONATES IN DETERGENTS**
Stephen W. Babulak and Lawrence Gildenberg, Colgate-Palmolive

**11:10** **17. SOAP-BASED DETERGENT FORMULATIONS. III: SURFACE ACTIVITY OF FATTY DERIVATIVES OF 3-HYDROXYPROPANE SULFONIC ACID**
N. Parris, J.K. Weil and W.M. Linfield, E. Market. Nutr. Res. Div., ARS, USDA

**11:30** **18. SOAP-BASED DETERGENT FORMULATIONS. IV: THE SYNTHESIS AND SURFACE ACTIVE PROPERTIES OF SULFOPROPYL ESTERS OF N-SUBSTITUTED IMINODIACETIC ACIDS**
W.M. Linfield, T.J. Micich, M.K. Sucharski and J.K. Weil, E. Market. Nutr. Res. Div., ARS, USDA

## MONDAY AFTERNOON—SEPTEMBER 25

**2:00 P.M.—Adam Room**

## SESSION F—SYMPOSIUM: NUTRITION OF 22-CARBON FATTY ACIDS

Chairman—**Joyce Beare-Rogers,** Dept. of National Health and Welfare, Ottawa, Ont., Canada

**2:00** **19. DIGESTION AND METABOLISM OF RAPESEED OIL BY THE HUMAN AND OTHER SPECIES**
Bruce E. McDonald, University of Manitoba

**3:30** **20. WHOLESOMENESS OF THE COMPLETE AND PARTIAL ESTERS OF FULLY HYDROGENATED RAPESEED OIL AS DETERMINED IN FEEDING STUDIES WITH RATS**
Fred H. Mattson and G. Nolen, Procter & Gamble Co.

**3:00** **21. BIOCHEMICAL EFFECTS OF DIETARY VERY LONG CHAIN FATTY ACIDS ON RAT HEART AND LIVER**
U.M.T. Houtsmuller, C.B. Struyk and A.v.d. Beek, Unilever

**3:30** **22. CRAMBE AND RAPESEED OIL CHRONIC TOXICITY STUDY IN RATS**
A.N. Booth, D.J. Robbins, M.R. Gumbann, D.H. Gould, W.H. Tallent and I.A. Wolff

## MONDAY AFTERNOON—SEPTEMBER 25

**2:00 P.M.—Convention Hall**

## SESSION G—SYMPOSIUM: I. LIPIDS OF MARINE MAMMALS

Chairman—**R.G. Ackman,** Fisheries Research Board of Canada, Halifax, N.S., Canada

**2:00** **INTRODUCTORY COMMENTS**
R.G. Ackman, Fisheries Research Board of Canada

**2:05** **23. LIPID COMPOSITION OF ERYTHROCYTES**

AND PLASMA FROM MARINE MAMMALS
G.J. Nelson, Lawrence Radiation Lab.

2:35 24. PHOSPHOLIPIDS OF ANTARCTIC SEI WHALE LIVER. FRACTIONATION BY SILVER NITRATE THIN LAYER CHROMATOGRAPHY
N.R. Bottino, Texas A&M University

3:05 25. DISTRIBUTION AND METABOLISM OF ORGANOCHLORINE PESTICIDES AND RELATED COMPOUNDS IN MARINE MAMMALS
R.F. Addison, Fisheries Research Board of Canada

3:35 26. CHARACTERIZATION OF UNUSUAL WAX ESTERS FROM THE JAW FAT OF THE ATLANTIC BOTTLE-NOSED DOLPHIN (TURSIOPS TRUNCATUS)
R.G. Ackman, J.C. Sipos, C. Litchfield and B. Hilaman, Fisheries Research Board of Canada and Rutgers University

4:05 27. COMPARATIVE LIPID PATTERNS IN THE HEAD FATS OF DOLPHINS, PORPOISES AND TOOTHED WHALES
C. Litchfield and A.M. Greenberg, Rutgers University

4:30 28. STUDIES OF SEAL AND FETAL WHALE LIPIDS
L.L. Gershbein and R.G. Ackman, Northwest Institute for Medical Research and Fisheries Research Board of Canada

## MONDAY AFTERNOON—SEPTEMBER 25
**2:00 P.M.—Banquet Room**

## SESSION H—SYMPOSIUM: PROCESSING OF EDIBLE OILS

Chairman—**T.K. Mag**, Canada Packers Ltd., Toronto, Ont., Canada

2:00 29. KINETICAL AND REACTION ENGINEERING ASPECTS OF THE FAT HYDROGENATION PROCESS
N.H. Schoon, Chalmers University of Technology

2:30 30. KINETICS OF HYDROGENATION OF VEGETABLE OILS
Sava Stefanovic, Lyle F. Albright and Robert H. Price, University of Cincinnati

3:00 31. DEUTERATION OF METHYL LINOLEATE WITH NICKEL, PALLADIUM, PLATINUM AND COPPER-CHROMITE CATALYSTS
Sambasevarao Koritala, E. Selke and H.J. Dutton, N. Reg. Res. Lab.

3:30 32. STACHIOMETRY OF COTTONSEED EXTRACTION
William H. King, S. Reg. Res. Lab.

4:00 33. WATER-RECYCLE WASHING OF REFINED SOYBEAN OIL: PLANT SCALE EVALUATION
R.E. Beal, L.T. Black and E.L. Griffin, N. Reg. Res. Lab., and J.C. Meng and G.S. Farmer, Anderson Clayton Foods

4:30 34. THE TESTING OF COMMERCIAL HYDROGENATION CATALYSTS
R.R. Allen and J.E. Covey, Anderson Clayton Foods

## MONDAY AFTERNOON—SEPTEMBER 25
**2:00 P.M.—Salle Richelieu**

## SESSION I—SYMPOSIUM: POLLUTION VIA EFFLUENT

Chairman—**K. Shikaze**, Environment Canada, Ottawa, Ont., Canada

2:00 35. FATTY WASTE PRODUCT UTILIZATION IN THE FOOD INDUSTRY
L. Emard, Laval University

2:30 36. A WATER POLLUTION CONTROL PROGRAM TYPICAL OF THE MEAT PACKING INDUSTRY
G.S. Moss and G.H. Kestle, J.M. Schneider Ltd.

3:00 37. REMOVAL AND RECOVERY OF FATTY MATERIAL FROM EDIBLE OIL AND FAT AND OIL REFINERY EFFLUENTS
W.C. Seng, Swift & Co.

3:30 38. ADVANCED PROCESS SYSTEMS FOR THE CONSERVATION, RECLAMATION AND REUSE OF SOLIDS AND WATER—THE SOLUTION TO POLLUTION
R.A. Gallop, University of Manitoba

## MONDAY AFTERNOON—SEPTEMBER 25
**2:00 P.M.—Renaissance Room**

## SESSION J—GENERAL BIOCHEMISTRY

Chairman—**Arnis Kuksis**, Banting and Best Dept. of Medical Research, Toronto, Ont., Canada

2:00 39. EFFECTS OF DIETARY POLYUNSATURATED FATTY ACIDS UPON TAIL DEVELOPMENT AND LIVER LIPID COMPOSITION OF IMMATURE NORMAL AND HYPOPHYSECTOMIZED RATS
O.S. Privett and E.W. Haeffner, The Hormel Institute

2:20 40. NATURE OF STEROLS AND STERYL ESTERS IN AORTA AND PLASMA OF ATHEROSCLEROSIS-SUSCEPTIBLE WHITE CARNEAU PIGEONS ON CHOLESTEROL-FREE DIETS
M.T. Ravi Subbiah, Mayo Clinic

2:40 41. DIETARY FACTORS AND AFLATOXIN TOXICITY
Roslyn B. Alfin-Slater, Lilla Aftergood, Penny Wells and Daniel Melnick, University of California at Los Angeles

3:00 42. GLYCERIDE METABOLISM IN ISOLATED MUCOSAL CELLS
P.J.A. O'Doherty, I.M. Yousef and A. Kuksis, Banting and Best Dept. of Medical Research

3:20 43. GLYCOLIPIDS OF PERIPHERAL NERVE: ISOLATION AND CHARACTERIZATION OF GLYCOLIPIDS FROM RABBIT SCIATIC NERVE
Harbhajan Singh, Dept. of Medicine, New York University

3:40 44. SYNTHETIC TRIGLYCERIDES AS STEREOSPECIFIC PROBES IN ENZYMIC LIPOLYSIS
N. Morley, A. Kuksis and D. Buchnea, Banting and Best Institute of Medical Research

4:00 45. PALMITYL COA REDUCTASE, PALMITALDEHYDE REDUCTASE AND PLASMALOGEN BIOSYNTHESIS IN CLOSTRIDIUM BUTYRICUM
Per-Otto Hagen and Lizzie Barber, Duke University Medical Center

4:20 46. DISTRIBUTION OF L-SERINE-$C^{14}$ AMONG MOLECULAR SPECIES OF GLYCEROPHOSPHOLIPIDS
S.K.F. Yeung and A. Kuksis, Banting and Best Dept. of Medical Research

4:40 47. LIPIDS AND FATTY ACIDS OF THE SOUTHWESTERN CORN BORER, DIATRAE GRANDIOSELLA
A.C. Thompson, Frank M. Davis, R.D. Henson, R.C. Gueldner, P.A. Hedin and C.A. Henderson, USDA

## TUESDAY MORNING—SEPTEMBER 26
**9:00 A.M.—Adam Room**

## SESSION K—SYMPOSIUM: RAPESEED MARKETING AND BREEDING

Chairman—**Keith Downey**, Canada Dept. of Agriculture, Saskatoon, Sask., Canada

9:00 48. THE EFFECT OF ENVIRONMENT AND GENETICS ON ERUCIC ACID IN COMMERCIAL CANADA RAPESEED
B. Craig, D. Irvine, Whyte, J.R. Reynolds and K. Downey, Canada Dept. of Agriculture

9:20 49. SELECTION FOR LINOLEIC AND LINOLENIC ACID IN RAPESEED
Gerhard Rakow, Canada Dept. of Agriculture

9:50 50. PLACE OF RAPESEED IN THE EDIBLE OIL MARKET
James McAnsh, Rapeseed Association of Canada

10:20 51. THE LIPID BIOCHEMISTRY AND ULTRA-

STRUCTURE OF DEVELOPED RAPESEED
L.-A. Appelqvist, University of Lund

10:50 52. COMPARISON OF CHEMICAL AND AGRONOMIC CHARACTERISTICS OF THE SPRING RAPE (BRASSICA NAPUS) VARIETIES BRONOWSKI AND TARGET
A.J. Finlayson, J. Krzymanski and R.K. Downey

11:10 53. VARIETAL DIFFERENCES IN PROTEIN OF ORIENTAL MUSTARD (B. JUNCEA L.)
S.L. MacKenzie

## TUESDAY MORNING—SEPTEMBER 26

**9:00 A.M.—Convention Hall**

## SESSION L—SYMPOSIUM: II. MARINE INVERTEBRATES

Chairman—**Carter Litchfield**, Rutgers University, New Brunswick, N.J.

9:00 54. BIOSYNTHESIS OF LIPIDS IN THE MARINE COPEPOD, EUCHAETA NORVEGICA
J.R. Sargent, R.R. Gatten and R. McIntosh, Institute of Marine Biochemistry

9:20 55. A UNIQUE LYSOPHOSPHOLIPID IN MARINE COPEPODS
R.F. Lee, S. Patton and A.A. Benson, Pennsylvania State University and Scripps Institute of Oceanography

9:40 56. LIPIDS OF MARINE CRUSTACEANS
J.C. Nevenzel and N.K. Menon, University of California at Los Angeles

10:00 57. SPHINGOLIPIDS IN MARINE SHELLFISH
A. Hayashi, T. Matsubara and F. Matsuura, Kinki University

10:20 58. PHOSPHONOSPHINGOLIPIDS OF SHELLFISH
T. Hori, O. Itasaka, M. Sugita and M. Iwamori, Shiga University

10:40 59. STUDIES ON THE DIGESTIVE LIPASE OF THE SURF CLAM, SPISULA SOLIDISSIMA
J.S. Patton and J.G. Quinn, University of Rhode Island

11:00 60. COMPARATIVE BIOCHEMISTRY OF JELLYFISH: FATTY ACIDS OF SCYPHOZOAN POLYPS AND MEDUSAE AND THE EFFECT OF DIET ON FATTY ACID COMPOSITION
J.D. Joseph, P.L. Zubkoff and E.B. Joseph, South Carolina Marine Research Laboratory and Virginia Institute of Marine Science

11:20 61. NONMETHYLENEINTERRUPTED POLYETHYLENIC FATTY ACIDS IN MARINE INVERTEBRATES
M. Paradis and R.G. Ackman, Fisheries Research Board of Canada

11:40 62. A UNIQUE ROLE FOR DOCOSAHEXAENOATE IN FISH
Ian J. Tinsley, Oregon State University

## TUESDAY MORNING—SEPTEMBER 26

**9:00 A.M.—Banquet Room**

## SESSION M—WHAT OSHA REGULATIONS MEAN TO THE OILSEED INDUSTRY

Chairman—**C.L. Kingsbaker**, Blaw-Knox Chemical Plants Inc., Pittsburgh, Pa.

## TUESDAY MORNING—SEPTEMBER 26

**9:00 A.M.—Salle Richelieu**

## SESSION N—SYMPOSIUM: LIPID INVOLVEMENT IN TOXICITY OF PESTICIDES AND OTHER DRUGS

Chairman—**A. de Freitas**, National Research Council of Canada, Ottawa, Ont., Canada

9:00 63. LIPID METABOLISM IN RELATION TO ABSORPTION, TRANSPORT, STORAGE AND MOBILIZATION IN MAMMALS: A REVIEW
Jean Himms-Hagen, University of Ottawa

9:30 64. ROLE OF LIPIDS IN DRUG DETOXICATION
R.M. Welch, Medicinal Biochemistry, The Wellcome Research Labs., Burrought Wellcome Co.

10:00 65. MOBILITY OF LIPOPHILIC PESTICIDES IN AN ANIMAL
G.M. Findlay, Dept. of Entomology, University of Manitoba

10:30 66. MODES OF ACTION OF LIPOPHILIC PESTICIDES AT THE SUBCELLULAR LEVEL
W. Chefurka, Research Institute, Canada Agriculture

11:00 67. THE IMPORTANCE OF BEING LIPID SOLUBLE
D.J. Ecobichon, Dept. of Pharmacology, Dalhousie University

## TUESDAY MORNING—SEPTEMBER 26

**9:00 A.M.—Renaissance Room**

## SESSION O—SYMPOSIUM: MILK LIPIDS

Chairman—**Lloyd M. Smith**, University of California, Davis, California

9:00 68. INTRODUCTION (INCLUDING OCCURRENCE OF MILK FAT IN DIFFERENT FOODS AND ITS ECONOMIC IMPORTANCE)
Lloyd M. Smith, University of California at Davis

9:20 69. ORIGIN OF THE MILK FAT GLOBULE (INCLUDING SYNTHESIS AND RELEASE. STRUCTURE OF MILK FAT GLOBULE MEMBRANE)
Stuart Patton, Pennsylvania State University

10:00 70. COMPOSITION OF MILK LIPIDS (INCLUDING CLASSES OF LIPIDS PRESENT AND FATTY ACID COMPOSITION OF GLYCERIDES AND PHOSPHOLIPIDS)
Robert G. Jensen, University of Connecticut

10:40 71. TRIGLYCERIDE STRUCTURE OF MILK LIPIDS
Arnis Kuksis, University of Toronto

## TUESDAY AFTERNOON—SEPTEMBER 26

**2:00 P.M.—Adam Room**

## SESSION P—SYMPOSIUM: PROCESSING OF RAPESEED AND RAPESEED OIL

Chairman—**Berd. Weinberg**, Dept. of Industry, Trade and Commerce, Ottawa, Ont., Canada

2:00 72. CONTROL OF CHLOROPHYLL CONTENT IN SWEDISH RAPESEED AND ITS IMPORTANCE FOR THE QUALITY OF THE OIL
J. Dahlon, *Swedish Oil Extraction Co. Ltd.*

2:30 73. GLUCOSINOLATE-FREE FLOURS AND ISOLATES FROM RAPESEED AND RELATED SPECIES
H. Kozlowska and F.W. Sosulski, Dept. of Crop Science, University of Saskatchewan

## TUESDAY AFTERNOON—SEPTEMBER 26

**2:00 P.M.—Convention Hall**

## SESSION Q—SYMPOSIUM: III. AQUATIC FOOD CHAINS

Chairman—**J.R. Sargent**, Institute of Marine Biochemistry

2:00 74. USE OF FATTY ACID PATTERNS TO DETERMINE FEEDING RELATIONSHIPS IN THE SEA
H.P. Jeffries, University of Rhode Island

2:20 75. JEDDORE HARBOUR AND ITS FATTY ACIDS: RESULTS OF A SURVEY FOR HYDROCARBONS AND FATTY ACIDS IN THE WATER AND AN EXPLANATION FOR THE ODD CHAIN FATTY ACIDS FOUND IN SMELT
M. Paradis and R.G. Ackman, Fisheries Research Board of Canada

2:40 76. FATTY ACIDS IN NATURALLY OCCURRING ORGANISMS AND TISSUES OF RAINBOW TROUT PLANTED IN AN ALKALINE PRAIRIE LAKE IN CANADA
M. Yurkowski, J. Tabachek and H. Boese, Fisheries Research Board of Canada

3:00 77. FATTY ACIDS IN AN INTERTIDAL POLY-

CHAETE: THEIR RELATION TO THE MARINE FOOD CHAIN AND TO THE PHYSIOLOGY OF THE WORM
D.M.E. Pocock, J.G. Hamilton and J.R. Marsden, McGill University and Hoffmann-LaRoche

## SYMPOSIUM: IV. FISH

3:20 78. 7-METHYL-7-HEXADECENOIC ACID IN THE OCEAN SUNFISH MOLA MOLA
R.G. Ackman, S.N. Hooper and M. Paradis, Fisheries Research Board of Canada

3:40 79. COMPARATIVE FATTY ACIDS OF PACIFIC TELIOST FISH
J.B. Saddler, Mississippi State University

4:00 80. MONOETHYLENIC ISOMERS IN CARDIAC LIPIDS OF RATS FED PARTIALLY HYDROGENATED HERRING OIL
H.B.S. Conacher and J. Beare-Rogers, Canadian Dept. of National Health and Welfare

4.20 81. WAX ESTERS IN BARRACUDINA
R.G. Ackman, C.A. Easton and S.N. Hooper, Fisheries Research Board of Canada

4:40 82. PHOSPHOLIPID ACTIVITY IN THE BRAIN OF A POLYCHAETE WORM, NEREIS VIRENS
Joan Marsden, McGill University

## TUESDAY AFTERNOON—SEPTEMBER 26

**2:00 P.M.—Banquet Room**

## SESSION R—SYMPOSIUM: NEW ADVANCES IN ANALYTICAL TECHNIQUES

Chairman—**S.F. Herb**, ARS, USDA, Philadelphia, Pa.

2:00 83. PROGRESS IN THE STRUCTURAL ELUCIDATION OF FATTY ACIDS AND TRIGLYCERIDES THROUGH THE USE OF CHEMICAL SHIFT REAGENTS AND NMR SPECTROSCOPY
Philip E. Pfeffer, USDA

2:30 84. COMPUTER IDENTIFICATION OF FATS AND OILS FROM THEIR FATTY ACID COMPOSITION
S.F. Herb and Paul J. Gormisky, USDA

3:00 85. MICROPARTICULATE BED CHROMATOGRAPHY ACCELERATED BY CENTRIFUGAL FORCE AND PRESSURE
Edgar Ribi, Rocky Mountain Lab.

3:30 86. IDENTIFICATION OF LIPIDS FROM THE VAPOR PHASE THERMAL FRAGMENTATION PATTERNS OF GAS LIQUID CHROMATOGRAPHY EFFLUENTS
Eugene J. Levy, Chemical Data Systems

## TUESDAY AFTERNOON—SEPTEMBER 26

**2:00 P.M.—Salle Richelieu Room**

## SESSION S—LIPOPROTEINS

Chairman—**W.G. Martin**, National Research Council of Canada, Ottawa, Ont., Canada

2:00 87. EXCHANGE OF LIPOPROTEIN CHOLESTEROL WITH INTACT AORTIC TISSUE AND ISOLATED ERYTHROCYTE MEMBRANES
Frank P. Bell, McMaster University

2:20 88. THE EFFECTS OF DIETARY CHOLESTEROL ON THE SYNTHESIS OR RELEASE OF RAT SERUM HIGH DENSITY LIPOPROTEINS
Jerome V. Franka and Raymond Reiser, Texas A&M University

2:40 89. QUANTITATION OF HUMAN PLASMA LOW DENSITY LIPOPROTEINS BY SINGLE RADIAL IMMUNODIFFUSION OF WHOLE PLASMA
Richard J. King and Joe C. Christian, Indiana University Medical School

3:00 90. DYNAMICS OF CHOLESTEROL AND CHOLESTERYL ESTERS IN THE LIVER PERFUSION SYSTEM
Sandra J. Petersburg and R.D. Ellefson, Mayo Clinic and Mayo Graduate School of Medicine

3:20 91. INVESTIGATION OF HIGH-DENSITY SERUM LIPOPROTEINS BY FLUORESCENCE AND FLUORESCENT PROBES
Norman K. Freeman and Alex V. Nichols, Donner Lab., University of California at Berkeley

3:40 92. THERMOGRAVIMETRIC ANALYSIS OF SERUM LIPOPROTEIN FRACTIONS
Frank T. Lindgren and Gary R. Stevens, Donner Lab., University of California at Berkeley

4:00 93. A RAPID SCREENING TEST FOR ELEVATED SERUM TRIGLYCERIDES OR CHOLESTEROL, OR BOTH
Joe C. Christian, Indiana University Medical School

## TUESDAY AFTERNOON—SEPTEMBER 26

**2:00 P.M.—Renaissance Room**

## SESSION T—SYMPOSIUM: MILK LIPIDS

2:00 INTRODUCTORY REMARKS
Lloyd M. Smith, University of California at Davis

2:05 94. CRYSTALLIZATION AND COMMERCIAL FRACTIONATION OF MILK FAT
John W. Sherbon, Cornell University

2:40 95. SOME FACTORS AFFECTING HYDROGENATION OF MILK FAT
Lloyd M. Smith and Andres Vasconcellos, University of California at Davis

3:00 96. METHODS FOR THE ISOLATION AND CHARACTERIZATION OF TRACE COMPONENTS FROM MILKFAT
Dan P. Schwartz, E. Utiliz. Res. Dev. Div., USDA

3:40 97. NUTRITIONAL VALUE OF FATS
Fred H. Mattson, Procter and Gamble Co.

4:20 DISCUSSION AND CONCLUDING REMARKS

## WEDNESDAY MORNING—SEPTEMBER 27

**9:00 A.M.—Adam Room**

## SESSION U—SYMPOSIUM: FOOD LIPIDS

Chairman—**Rene Riel**, Laval University, Queb., Canada

9:00 98. THE ROLE OF FOOD LIPIDS IN HUMAN NUTRITION
M.A. Amer and G.J. Brisson, Nutrition Research Center, Laval University

9:30 99. CONTROL OF BLOOD LIPID LEVELS
Paul-J. Lupien, Lipid Disease Research Center, Center Hospital, Laval University

10:00 100. EFFECT OF DIET ON THE COMPOSITION OF BODY FAT
Germain J. Brisson, Nutrition Research Center, Laval University

10:30 101. MICROBIAL LIPIDS
J. Goulet and A.C. Blackwood, Macdonald College of McGill University

11:00 102. EVALUATION OF A GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF FOOD TOCOPHEROLS
Hal T. Slover and Joanne Lehmann, Human Nutrition Research Div., Agricultural Research Institute, Beltsville, Md.

## WEDNESDAY MORNING—SEPTEMBER 27

**9:00 A.M.—Convention Hall**

## SESSION V—SYMPOSIUM: MEMBRANE LIPIDS

Chairman—Pierre Proulx, University of Ottawa, Ottawa, Ont., Canada

9:00 103. INTERPRETATION OF THE ELECTRON SPIN RESONANCE SPECTRA OF LIPID SPIN PROBES IN HYDRATED LIPID MULTIBILAYERS
Ian C.P. Smith, K.W. Butler, R.D. Lapper, S. Schreier-Muccillo and D. Marsh, National Research Council of Canada

9:30 104. **SPIN LABEL STUDIES OF MEMBRANE STRUCTURE**
J.C. Hsia, University of Toronto

10:00 105. **THE NATURE OF MEMBRANOUS FRACTIONS DERIVED FROM YEAST PROTOPLASTS**
J.B.M. Rattray, University of Guelph

10:30 106. **MITOCHONDRIAL AUTONOMY IN THE BIOSYNTHESIS OF POLYGLYCEROPHOSPHATIDES**
N.Z. Stanacev, Banting Institute, University of Toronto

## WEDNESDAY MORNING—SEPTEMBER 27

**9:00 A.M.—Banquet Room**

## SESSION W—SYMPOSIUM: PLASTICITY OF LIPIDS

Chairman—**John de Man**, University of Guelph, Guelph, Ont., Canada

9:00 107. **FAT MELTING POINT DETERMINATIONS: A REVIEW**
W.G. Mertens, Canada Packers Ltd.

9:30 108. **FAT POLYMORPHISM**
E.S. Lutton

10:00 109. **DIELECTRIC BEHAVIOR OF TRIPALMITIN AND TRISTEARIN**
G.W. Hoerr and F.R. Paulicka, Glidden Durkee, Div. of SCM Corp.

10:30 110. **RHEOLOGICAL PROPERTIES OF HARD BUTTERS**
F.R. Paulicka and T.J. Bansek, Glidden-Durkee, Div. of SCM Corp.

11:00 111. **DETERMINATION OF THE SOLIDS CONTENT IN PARTLY CRYSTALLIZED FATS**
Adolf J. Haighton, Unilever Research Lab.

## WEDNESDAY MORNING—SEPTEMBER 27

**9:00 A.M.—Salle Richelieu**

## SESSION X—GENERAL

Chairman—**C.Y. Hopkins**, Ottawa, Ont., Canada

9:00 112. **A COMPARISON OF THE WIDELINE AND PULSED NMR METHODS FOR DETERMINING THE SOLID-LIQUID RATIOS IN FATS**
Gareth J. Templeman and Eng C. Wang, The Pillsbury Co.

9:20 113. **COMPARATIVE STUDY OF THE EUTECTIC BODIED OIL WITH STAND OIL BY NMR SPECTRAL ANALYSES**
S.N. Koley

9:40 114. **THERMAL ALTERATION OF FATTY ACIDS AND THEIR DERIVATIVES IN THE PRESENCE OF MINERAL CATALYSTS**
A. Eisner, T.A. Foglia and I. Schmeltz, USDA

10:00 115. **CATALYTIC HYDROCARBOXYLATION OF FATS. POLYCARBOXY ACIDS FROM MONO- AND POLYUNSATURATES**
E.N. Frankel and F.L. Thomas, N. Reg. Res. Lab.

10:20 116. **POLY (AMIDE-ACETALS) AND POLY (ESTER-ACETALS) FROM PENTAERYTHRITOL ACETALS OF METHYL 9(10)-FORMYLSTEARATE CROSS-LINKED STATIONARY PHASES FOR GAS CHROMATOGRAPHY**
J.C. Cowan, W.E. Neff, R.A. Awl and E.H. Pryde, N. Utiliz. Res. Dev. Div.

10:40 117. **NMR CHEMICAL SHIFT REAGENTS IN STRUCTURAL DETERMINATION OF LIPID DERIVATIVES. IV: METHYL 9,10-DIHYDROXY- AND EPOXYSTEARATE**
John P. Wineburg and Daniel Swern

11:00 118. **THE EFFECTS OF AMMONIATION ON AFLATOXINS IN RATIONS FED LACTATING COWS**
John D. McKinney and George C. Cavanagh, Ranchers Cotton Oil

11:20 119. **HEAT INACTIVATION OF TRYPSIN INHIBITOR AND SOYBEAN ENZYME—THE EFFECT OF ACID AND BASE ADDITIVES**
E.C. Baker and G.C. Mustakas, N. Reg. Res. Lab.

11:40 120. **HONORED STUDENT PRESENTATION**
**CREAMING IN EVAPORATED MILK**
K. Sabbarival and P.M.T. Hansen, Dept. of Food Science and Nutrition, Ohio State University

## WEDNESDAY MORNING—SEPTEMBER 27

**9:00 A.M.—Renaissance Room**

## SESSION Y—BACTERIAL FUNGAL AND PLANT LIPIDS

Chairman—**Morris Kates**, University of Ottawa, Ottawa, Ont., Canada

9:00 121. **DETECTION OF SULFATED CEREBROSIDES IN GLOMERELLA CINGULATA**
Gregory E. Anekwe and Linda L. Lee, Dept. of Chemistry, Tuskegee Institute

9:20 122. **CHANGES IN THE STRUCTURE OF TRIGLYCERIDES FROM MATURING KERNELS OF CORN (ZEA MAYS L.)**
Evelyn J. Weber, University of Illinois

9:40 123. **BIOSYNTHESIS OF PHOSPHOLIPIDS IN CELL-FREE EXTRACTS OF SPINACH LEAVES**
M.O. Marshall and Morris Kates, University of Ottawa

10:00 124. **HONORED STUDENT PRESENTATION**
**FATTY ACID 12- AND 15-DESATURATION BY PENCILLIUM CHRYSOGONUM Q176**
Roberta L. Richards and Forrest W. Quackenbush, Purdue University

10:20 125. **HONORED STUDENT PRESENTATION**
**THE ROLE OF MEMBRANE LIPIDS IN LOW TEMPERATURE ADAPTATION OF PLANTS**
Daryl G. Richardson and Conrad J. Weiser, University of Minnesota

10:40 126. **LEAF WAX OF PORTULACA OLERACEA L.**
A.P. Tulloch, National Research Council of Canada

11:00 127. **STEROSPECIFIC ANALYSIS OF TRIACYLGLYCEROLS FROM LIPOMYCES LIPOFERUS**
James E. Haley and R. Cecil Jack, St. John's University

11:20 128. **PHOSPHOLIPIDS OF ARTHRODERMA UNICINATUM**
Zoltan Kish and R. Cecil Jack, St. John's University

11:40 129. **LIPID COMPOSITION OF MORNING GLORY CELL SUSPENSION CULTURES. (IPOMEA *SP.*)**
Moonja Song and Neil Tattrie, National Research Council of Canada

## WEDNESDAY AFTERNOON—SEPTEMBER 27

**2:00 P.M.—Salle Richelieu**

## SESSION Z—GENERAL BIOCHEMISTRY

Chairman—**James Rattray**, University of Guelph, Guelph, Ont., Canada

2:00 130. **OXYGENATION OF UNSATURATED FATTY ACIDS IN SEEDS DURING STORAGE**
G.F. Spencer, F.R. Earle, I.A. Wolff and W.H. Tallent, ARS, USDA

2:20 131. **INFLUENCE OF DIETARY FATTY ACIDS ON THE LIVER MICROSOMAL FATTY ACID ACTIVATION OF CIS AND TRANS-Δ-9-OCTADECANOIC ACID IN VITRO**
Gustav Graff and F.A. Kummerow, Burnsides Research Lab., University of Illinois

2:40 132. **METABOLISM OF 1,2-DEHYDROXYHEPTADECANE AND 1-HYDROXY-2-KETOHEPTADECANE IN MAMMALIAN BRAIN**
H.H.O. Schmid and T. Muramatsu, Hormel Institute

3:00 133. **HUMAN PERIPHERAL NERVE MYELIN: QUALITATIVE AND QUANTITATIVE STUDIES WITH AGING**

Harbhajan Singh, Norton Spritz and Barbara Geyer, New York University School of Medicine, Veterans Administration Hospital

3:20 134. **INHIBITION OF DESATURATION AND ELONGATION OF FATTY ACIDS BY ACETYLENIC ACID**
H.C. Chang and Ralph T. Holman, Hormel Institute

3:40 135. **LONG CHAIN CYCLIC ACETALS OF GLYCEROL. A STRUCTURAL STUDY**
Wolfgang J. Baumann, Hormel Institute

4:00 136. **METABOLISM OF LONG CHAIN CYCLIC ACETALS OF GLYCEROL IN MAMMALIAN BRAIN**
K.L. Su, W.J. Baumann, T. Madson and H.H.O. Schmid, Hormel Institute

## WEDNESDAY AFTERNOON—SEPTEMBER 27

**2:00 P.M.—Renaissance Room**

## SESSION A-2—ANALYTICAL

Chairman—**A.P. Tulloch,** Prairie Reg. Lab., National Research Council of Canada, Saskatoon, Sask., Canada

2:00 137. **IMPROVED METHODS OF CONVERSION OF 9,10,12,13-DIEPOXYSTEARIC ACIDS TO 9,10,12, 13-TETRAHYDROXYSTEARIC ACIDS**
M.A. Khuddus, Y. Usui and Daniel Swern, Temple University

2:20 138. **AN AUTOMATED THERMOGRAVIMETRIC SYSTEM FOR THE MICRODETERMINATION OF LIPIDS**
Bipinchandra Patel, J. Leerkamp and A.N. Siakotos, Indiana University Medical Center

2:40 139. **HONORED STUDENT PRESENTATION**
**A STUDY OF THE MINOR CONSTITUENTS IN TALL OIL, FATTY ACIDS AND THE IDENTIFICATION OF TRANS-3,5-DIMETHOXY STILBENE**
David B.S. Min and Stephen S. Chang, Dept. of Food Science, Rutgers State University

3:00 140. **QUANTITATIVE ANALYSIS OF COMPLEX LIPID MIXTURES WITH A NEW SCANNER FOR THIN LAYER CHROMATOGRAPHY**
K.D. Mukherjee and H.K. Mangold, H.P. Kaufmann-Institut

3:20 141. **ASPECTS OF POROUS POLYMER BEADS FOR QUANTITATIVE ANALYSIS OF VOLATILE FATTY ACIDS AND ANOMALOUS PEAK BROADENING EFFECTS FOR METHYL-BRANCHED MATERIALS**
R.G. Ackman, Fisheries Research Board

# ABSTRACTS OF PAPERS

## 1

EMBRYONIC TISSUE LIPIDS: THE STATIC AND DYNAMIC STATES OF GLYCERIDES IN VARIOUS TISSUES DURING DEVELOPMENT. RANDALL WOOD and DANIEL H. WINSHIP, University of Missouri School of Medicine, Columbia, Mo. 65201.

Triglycerides were isolated from developing chick brain, heart and liver 10, 13, 16, 19, 22, 27 and 53 days after incubation had been initiated. Carbon number distributions were determined by high temperature gas liquid chromatographic analyses of the intact triglycerides. The percentage distribution of fatty acids derived from the triglycerides was also determined. All three issue triglycerides exhibited a wide range of carbon numbers (46–66) at the early development stages. The carbon number distribution and fatty acid composition of brain triglycerides remained unchanged throughout development, in contrast to heart and liver triglycerides, suggesting a lack of turnover. Comparison of calculated and determined carbon number percentages of brain triglycerides suggested a random distribution of fatty acids. The triglycerides of both heart and liver showed a dramatic decrease of high molecular weight species with increased developmental time. Comparison of determined and calculated carbon number percentages demonstrated a nonrandom distribution of fatty acids in liver triglycerides at all time periods, but surprisingly the heart triglyceride fatty acids approached a random distribution. Methyl esters derived from triglycerides of heart and liver showed a decrease in 20:4 and higher polyunsaturated fatty acids, especially 22:6, with increased development time. The decreased concentration of 22:6 corresponds to the decreased cell mitotic index suggesting the involvement of this acid in cell division. The data illustrate that the triglycerides of all three tissues differ markedly at all stages of development from egg triglycerides indicating de novo biosynthesis of the tissue triglycerides.

## 2

LIPIDS OF HUMAN TESTICULAR TISSUE. JOHN G. CONIGLIO and ROBERT K. RHAMY, Dept. of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tenn. 37232.

Testicular tissue obtained fresh at orchiectomy of patients (60–80 years of age) with prostatic cancer is being analyzed chemically for lipid and fatty acid content, and histologically with respect to the germinal epithelium. Average values obtained for various lipids were: total lipid (TL) 26 mg/g wet wt of tissue; total phosphatides, 64% of TL; total cholesterol, 7% of TL (ca. ⅔ free cholesterol); triglyceride, 18% of TL. About 60% of the total phosphatides was phosphatidyl choline; 8% sphingomyelin; 24% phosphatidyl ethanolamine; 6% phosphatidyl serine plus inositol; and 1% lysolecithin. Major fatty acids (as % of total fatty acids) included: 16:0, 32%; 18:0, 11%; 18:1, 18%; 18:2, 5%; 20:3, 5%; 20:4, 12%; 22:6, 7%. In general there was extensive degeneration of the germinal cells in these specimens. In two specimens obtained at autopsy of men of comparable age the content of lipid and of fatty acids was not significantly different from that described above. However in specimens obtained at autopsy of two infants dying soon after birth (25 and 58 hr old) the content of 20:3 and of 22:6 was remarkably low (less than 1% of the total fatty acids), although that of 20:4 (13 and 7%) was not.

## 3

UTERINE LIPID RESPONSE OF OVARIECTOMIZED RATS TO HORMONAL ADMINISTRATION. P.T. RUSSELL, BRUCE C. MOULTON and WILLIAM J. MILLER, University of Cincinnati Medical Center, Cincinnati, Ohio 45229.

The response of uterine lipid to human chorionic gonadotropin (HCG; 1 IU, ip) administered to rats ovariectomized 8 weeks prior to treatment consisted of an increased deposition of lipid, predominately triglyceride, and a significant shift in fatty acid composition (FAC) to the unsaturated fatty acids. The FAC changes observed were associated primarily with the triglycerides and free fatty acids. These FAC changes occurred at the same time as changes in lipid content which became maximum 2 hr after the administration of HCG. FAC analyses of total uterine lipid following estradiol or progesterone administration (1 μg for 5 days, s.c., and 5 mg for 3 days, s.c., respectively) to overiectomized rats demonstrated increased proportions of saturated fatty acids after estradiol and no significant change from controls after progesterone. Progesterone did not interfere with the FAC response to estradiol when administered in combination with estradiol. The lipid class changes seen after estradiol conformed to the known effects of estradiol on lipid metabolism. These observations suggest that the rapid, transient changes in uterine lipid content and FAC of normal cycling rats attending the time of ovulation can be mediated, in part, by gonadotropin.

## 4

IN VITRO HEPATIC CHOLESTEROGENESIS IN RATS FED CORN OIL AND FAT-FREE DIETS AND ITS INHIBITION BY EXOGENOUS LINOLEATE. P.O. EGWIM and F.A. KUMMEROW, Burnsides Research Lab., University of Illinois, Urbana, Ill. 61801.

Cholesterogenesis from mevalonate-2-$^{14}C$ and linoleate-1-$^{14}C$ was measured in vitro using hepatic $S_{40}$ preparations from male Holtzman strain rats fed, from weanling, either a 20% corn oil diet or a fat-free diet for a period of 6 months. Mevalonate was found to be a by far better substrate than linoleate in both dietary groups. Furthermore, cholesterogenesis from mevalonate was more efficient with the $S_{40}$ preparation from the corn oil-fed rats than with that from the fat-deficient animals. However, when the influence of increasing concentrations of exogenous linoleate (0–8.3 μM) on cholesterol synthesis from mevalonate was tested, no stimulatory effect was observed. It was found that while lower concentrations of linoleate did not seem to have any adverse effect, higher concentrations (1.6–8.3 μM, respectively, for the corn oil and fat-free groups) resulted in a 92% inhibition for the former, and an 84% inhibition for the latter group of rats. The relationship of these results to the widely reported enhancement of hepatic cholesterogenesis in linoleate-fed rats is discussed from the standpoint of possible indirect rather than direct effects of dietary linoleate, presumably involving some fatty acid compositional changes in the phospholipid moieties of the lipoprotein components of the relevant key enzymes.

## 5

EFFECT OF TERTIARY BUTYLHYDROQUINONE AND OTHER ANTIOXIDANTS ON OXIDATIVE STABILITY OF MARINE OILS. PART 1: WHALE OIL. M.H. CHAHINE and R.F. MACNEILL, Nova Scotia Research Foundation, 100 Fenwick St., P.O. Box 790, Dartmouth, N.S., Canada.

Crude whale oil was stored under accelerated conditions for 146 days alone and in the presence of added antioxidants: 0.02% butylated hydroxyanisole (BHA), 3,5-di-*t*-butyl-4-hydroxyanisole (Di-BHA), propyl gallate (PG), tertiary butylhydroquinone (TBHQ), TBHQ + 0.01% citric acid and 0.01% citric acid. Inverse relationships were noted between levels of peroxide value (PV) and oxidative stability time and also between secondary oxidation products, measured by anisidine value (AV), and oxidative stability time at termination of the storage period of the antioxidant-treated crudes. TBHQ gave best results with respect to retardment of oxidative deterioration. All antioxidant-treated crudes were processed further in the laboratory. An unstored crude oil was also processed under the same conditions. The improvement in oxidative stability due to decrease in level of secondary oxidation products was calculated from the difference in stability times between deodorized oils from unstored (AV:8) and stored (AV:58) crudes. Stability times of deodorized oils from crudes treated with BHA, Di-BHA, PG and citric acid ranged from 13.5–18 days at 80 C. Deodorized oils from TBHQ- and TBHQ-citric acid-treated crudes gave much longer stability times against oxidative deterioration at 80 C, viz., 49.5 and 56 stability days, respectively. Several factors contributed in various proportions to the extended induction period and caused the continued effectiveness of TBHQ even after deodorization. The original oil contributed 19.2% to stability time, and 13.1% was due to the relatively low level of secondary oxidation products. A residual 0.002% TBHQ in the deodorized oil contributed 10.1% of the stability time, and 57.6% was attributable to the *antioxidant potency* of the *oxidation products of TBHQ*. The oxidative stability of all deodorized oils from antioxidant-treated crudes was further improved by addition of 0.02% TBHQ.

## 6

DOCOSAHEXAENOATE BIOSYNTHESIS BY A HETEROTROPHIC, MARINE DINOFLAGELLATE. DAVID H. BEACH, Department of Microbiology, S.U.N.Y. Upstate Medical Center, Syracuse, N.Y. 13210.

The heterotrophic, marine dinoflagellate, *Crypthecodinium cohnii*, biosynthesizes only one polyunsaturated fatty acid in an amount greater than 1% of its total fatty acids; all-*cis*-4, 7, 10, 13, 16, 19-docosahexaenoic acid (22:6 ω3). In cells from cultures gassed with air, 22:6 ω3 was ~50% of the total fatty acids, ~25% of triglyceride fatty acids and ~65% of phosphatidylcholine fatty acids. Other unsaturates present included oleic acid (~10%), 5 positional isomers of 16:1 (total ~1%), 4 positional isomers of 14:1 (total <1%) and 22:5 ω3 (~1%). The monoenoic acids were found mainly in the triglyceride. The remaining fatty acids were saturated, straight chain, even-numbered, $C_{10}$-$C_{18}$ acids. The fatty acid composition of *C. cohnii* was related to the $O_2$ tension of the medium. Gassing with $N_2$ caused a rapid depression of triglyceride 22:6 ω3, 18:1 and 16:0, and an elevation of 10:0, 12:0, 14:0 and 18:0. No changes occurred in the phosphatidylcholine. Variations of temperature (15–30 C), culture age (1–10 days) and salinity (0.5–5%) influenced the fatty acid composition of the triglyceride only slightly and that of the phosphatidylcholine not at all. The constancy of the fatty acid composition of the main polar lipid, phosphatidylcholine (~75% of polar lipids), is thought to reflect the constraints placed upon the structure and function of this membrane lipid by the superabundance of 22:6 ω3. A molecule of 22:6 ω3 occupies one acylation site, on the average, on each molecule of the phospholipid. *C. cohnii* was exposed during growth in cultures gassed with air to $^{14}C$-labeled fatty acids which might be precursors of 22:6 ω3. Of these, 8:0 clearly introduced radioactivity into the carbon chain of the polyunsaturate. Results with 10:0 and 12:0 were equivocal, and the following were clearly inactive: 14:0, 16:0, 18:0, 9–18:1, 9,12–18:2, 6,9,12–18:3 and 9,12,15–18:3. Radioactivity was introduced, however, into 9–18:1 by all the $^{14}C$-labeled $C_8$-$C_{18}$ saturated acids. Randomization of labeling, by β-oxidation of the fatty acid and recycling of $C_2$ units, has been established for 8:0, is suggested for 10:0 and 12:0, and has been ruled out for the other fatty acids. The patterns of distribution of radioactivity obtained in these experiments suggest compartmentalization of 9–18:1 and 22:6 ω3 biosyntheses, and inaccessibility of the mechanism for biosynthesis of 22:6 ω3 to the exogenous $^{14}C$-labeled fatty acids. The exogenous acids and their conversion products were incorporated into the triglyceride and phosphatidylcholine fractions, however. It is inferred, therefore, that the transacylation enzymes were accessible.

## 7

THE PRACTICAL ASPECTS OF FATS & OILS IN FRYING. B.L. THOMAS and K.H. GOBERTEMILLER, B.L. Thomas Associates, Box 15177, Cincinnati, Ohio 45215.

The ever growing popularity of drive-in, fried chicken establishments has produced a specific study of the advantages and disadvantages of all commercial pressure fryers—particularly

with respect to their effect on the frying shortening itself, and the resultant carry-over effect on the finished fried chicken. Virtually all of the chicken so produced today is fried in "pressure pot" type cookers. However our study has been able to recognize no basic differences in the equipment available for such production, with one notable exception—a fryer which employs the most effective "cold zone" available. Furthermore this equipment is so "easy" on the frying shortening that rarely is it ever necessary to discard it. In spite of this, however, the objective and subjective tests of the shortening are superior to those found with any other similar type of equipment.

## 8

EFFECTS OF FEEDING TRIUNDECANOIN TO COWS AND RATS. JOEL BITMAN, R.W. MILLER, T.R. WRENN and L.P. DEYDEN, ARS, USDA, Dairy Cattle Research Branch, Beltsville, Md. 20705.

Medium chain triglycerides are degraded to 2-carbon fragments in vivo and subsequently utilized. During $\beta$-oxidation of an odd-numbered carbon chain, propionyl residues as well as acetyl are generated. Effects on milk fat, depot fat and carbohydrate metabolism were assessed by administration of a C-11 triglyceride, triundecanoin (TUD), or corn oil to cows and rats. Milk fat from cows fed TUD as 5% of the diet showed increases in 11:0 from 0.2% to as much as 6% of total fatty acids. Little change occurred in other odd-numbered fatty acids. There was a marked increase in milk fat 18:1, from 26–40%, as well as an increase in 18:0. There were compensatory decreases in 16:0, 14:0 and 12:0 acids. No changes were observed in plasma cholesterol, NEFA, triglycerides or in milk cholesterol. TUD produced a 30% decline in feed consumption and an 18% decrease in milk yield, with little change in milk fat percentage. Rat adipose tissue was enriched with large amounts of odd-numbered fatty acids by feeding TUD for 6 weeks. Odd-numbered longer saturated fatty acids appeared with a corresponding decrease in oleate and linoleate. Weight gains of TUD-fed rats were 90% of those of corn-oil fed rats when both were vitamin $B_{12}$-sufficient, but only 53% (males) and 67% (females) as much when both were vitamin $B_{12}$-deficient. This indicated that vitamin $B_{12}$ was required to metabolize and utilize odd-carbon fatty acids and propionate. When subjected to 96 hr starvation, the rats enriched with odd-carbon fatty acids, whether vitamin $B_{12}$ deficient or not, maintained their liver glycogen at a higher level than corn oil-fed rats.

## 9

TRENDS IN PROCESSING AND MANUFACTURING IN EDIBLE OIL REFINERIES. BEN W. MINSHEW, Archer Daniels Midland Co., P.O. Box 1470, Decatur, Ill. 62525.

*Consumption of oils* is increasing and will continue in the foreseeable future. The history of refineries has been to locate in the vicinity of the oil source. Development in recent years in building and expansion of refineries has been largely in the oilseed belt, following the trend dating back to the refineries of the meat fat processors. With 80% of vegetable shortening and oils from a domestic oil, on the surface, this is logical. With modern processing equipment and automation, maximum production volume and quality indicate successful operations. A large volume refinery does not operate entirely on a readily available single source oil. Distant domestic and import oils play important roles in refineries due to available world sources of oils for economic and customer requirements. The interchangeability of domestic oils of differing geographical locations plus the interchangeability of imported oils in shortening blending and processing have had a far-reaching impact on refineries, the use of shortening and oils, and future developments of refineries for multipurpose oils. Refineries encourage repacking fats and oils in areas of geographical distribution, since this brings together fats and oils from varying locations for processing for shipment in bulk units. The refinery capacities have increased far in excess above required needs. Presently we believe the existing refinery capacities will cover shortening and oil requirements for consumer, commercial and institutional uses for the next decade.

## 10

CLAY-HEAT REFINING OF EDIBLE OILS. T.K. MAG, Canada Packers Ltd., 2211 St. Clair Avenue West, Toronto 167, Ont., Canada.

The treatment of crude edible oils with sodium hydroxide solutions is the standard refining procedure in the industry. Refining with NaOH removes free fatty acids, some phosphatides, proteinaceous matter and some colored material. Up to now experience has shown that most oils cannot be deodorized satisfactorily unless they have been caustic-refined. In the past, when most crude oils contained several per cent of free fatty acids, caustic-refining offered itself as a particularly suitable means of preparation for further processing. In recent years the free fatty acid content of crude oils has been, in most cases, only a fraction of 1%, which could very readily be removed in the process of deodorization. A prerequisite for this would be to remove by some other means those substances which interfere with satisfactory deodorizing. It would be particularly advantageous if this could be done in the process of bleaching, since this is necessary in any case to remove color. It has been found that the process of bleaching can be used for this purpose if the oil is pretreated with 0.1–0.5% phosphoric acid and bleached at 325–350 F. Without a phosphoric acid pretreatment, still higher bleaching temperatures, 425–450 F, are needed to achieve satisfactory results. The amount of bleaching clay required depends on the type of oil and its quality, but with many oils up to 2% clay is satisfactory. The amount of phosphoric acid necessary also depends on the type of oil.

## 11

APPLICATION OF PARTIAL HYDROGENATION THEORY TO THE DESIGN OF COMMERCIAL REACTORS FOR HYDROGENATING TRIGLYCERIDE OILS. LYLE F. ALBRIGHT, School of Chemical Engineering, Purdue University, Lafayette, Ind. 47907.

An understanding of the fundamentals of hydrogenation is required for optimum design of commercial hydrogenation reactors. The overall hydrogenation sequence always includes both positional and geometrical isomerization steps of major importance. Several key physical steps frequently control the character of the hydrogenation obtained, and these steps include dissolving the hydrogen in the oil (or liquid) phase and transfer of reactants to the catalyst surface. Adsorption steps plus chemical steps then occur on the catalyst surface. The fundamentals of hydrogenation are reviewed, and critical analyses are then made of several types of reactors including flow reactors.

## 12

THE CHEMICAL ANALYSIS OF POLYMERIZATION PRODUCTS IN ABUSED FATS AND OILS. ARTHUR E. WALTKING, WILLIAM E. SEERY and GEORGE W. BLEFFERT, CPC International, Inc.

For more than a decade a variety of analytical methods have been proposed for the detection and measurement of polymers in vegetable fats and oils. Many of the methods have been little more than laboratory curiosities either because they were concerned with only very specific compounds or were too cumbersome and time consuming to become very popular. More recently a number of methods in common use for analysis of fats and oils have been shown to be useful for indirectly measuring polymeric materials in heat-abused oils. The present report will compare in depth the limitations and the potential errors of a variety of these methods. It will also show, through the use of gel filtration chromatography, the validity of the indirect estimation of polymeric products of abused fats and oils through determination of the retention of such materials on a gas liquid chromatographic (GLC) column as well as through changes in the iodine value. A new simplified internal standard GLC procedure utilizing triglyceride standards will also be presented. This latter method permits estimating the degree of degradation of vegetable fats and oils by any laboratory capable of determining the fatty acid composition of a sample by GLC.

## 13

THE APPLICATION OF GEL PERMEATION CHROMATOGRAPHY TO HEATED FATS. E.G. PERKINS, R. TAUBOLD and A. HSEIH, Burnsides Research Lab., University of Illinois, Urbana, Ill. 61801.

Gel permeation chromatography has been widely employed for the separation of biologically important components, such as proteins. It has, however, been used to determine the molecular size of lipids only to a limited extent. By using lipophilic gels, high molecular weight oxidation products of fats may be separated. The separation characteristics of lipids in general and heated fats using the gels LH-20 and Bio-Beads-SX2 were investigated using acetone, chloroform, tetrahydrofuran and chloroform-methanol mixtures as the eluting solvents. As shown by plots of distribution coefficient ($K_{AV}$) v. logarithm of molecular weight, separation on the different columns varied. In general, baseline separation of monomers from dimers or polymers was obtainable. But satisfactory separation among dimers and polymers is difficult. A comparison of results from large (1.25 × 100 cm) column with small, high speed, high efficiency columns will be presented. Fractions from the columns were collected and analyzed by thin layer chromatography, gas chromatography, mass spectrometry and also elemental analysis.

## 14

THE PHOTO-DIMERIZATION PRODUCTS OF UNSATURATED FATTY ACID METHYL ESTERS. OSAMU SUZUKI and TETSUTARO HASHIMOTO, Government Chemical Industrial Research Institute, Hon-machi, Shibuya-ku, Tokyo, Japan.

The photo-dimerization of unsaturated fatty acid methyl esters was studied as a new method for producing the dimer acid. When irradiated with the light of the mercury lamp, the fatty acid methyl esters, especially the conjugated fatty esters, were observed to form dimer in solution in several solvents. The content of the dimer formed by the photo-reaction was determined by thin layer chromatography and temperature programed gas liquid chromatography. Then the dimer fractions separated by column chromatography were analyzed by IR, NMR and mass spectroscopy. This study showed that the yields of the dimers obtained by illumination with light in $CCl_4$ amount to ca. 50%. The photo-dimerization of the fatty esters is also proceeded in *n*-heptane, but the yields of the dimers are not so high. From the results of analyses of the products, the photo-reaction dimers in $CCl_4$ and in $CHCl_3$ were found to be chlorinated.

## 15

VARIABLES IN THE POWDERING OF FLOOR FINISHES. M.E. GINN and E.T. FRONCZAK, Masury-Columbia Co., 1502 N. 25th Ave., Melrose Park, Ill. 60160.

Powdering of floor polishes represents the breakdown of a film on the floor which occurs particularly under cold, low humidity conditions, and this has presented a serious problem to the industry. Factors in the powdering or dusting of floor polish films are reviewed. Variables include the effect of measurement technique, humidity, ambient temperature, floor tile substrates and polymer film forming temperature. Correlation between laboratory and field test results is presented, and high correlation is evident provided that extraneous factors such as soil residues are eliminated.

## 16

*AUTOMATED DETERMINATION OF SILICATES AND CARBONATES IN DETERGENTS.* STEPHEN W. BABULAK and LAWRENCE GILDENBERG, Colgate-Palmolive.

The analysis of silicate and carbonate builders in detergents is time consuming by standard gravimetric procedures. By modifying established AutoAnalyzer methods, rapid and simple methods of analyzing these detergent builders have been achieved. Standard AutoAnalyzer modules, arranged for colorimetric analysis, are used and 10–15 samples per hour can be handled. The methods are applicable for concentration ranges from ppm to the assaying of raw materials. In the silicate analysis, a sample dissolved in water is reacted with *ammonium molybdate in an acid medium to form silicomolybdic* acid. This acid is partially reduced by ascorbic acid forming the pentavalent molybdenum blue complex. The intensity of this color complex is directly proportional to the concentration

of silicate. The coefficient of variation of the method is 2.6%. This silicate method is subject to minor interference by orthophosphate, and a correction factor can be employed. The carbonate method is based upon the evolution of carbon dioxide gas which is reacted with a buffered phenolphthalein solution. The color intensity of the system is inversely proportional to the carbon dioxide concentration. The coefficient of variation is 1.0%. When a blend of carbonate and bicarbonate is present in the sample, analysis before and after heating is required.

## 17

SOAP-BASED DETERGENT FORMULATIONS. III: SURFACE ACTIVITY OF FATTY DERIVATIVES OF 3-HYDROXYPROPANESULFONIC ACID. N. PARRIS, J.K. WEIL and W.M. LINFIELD, E. Market. Nutr. Res. Div., ARS, USDA, 600 East Mermaid Lane, Philadelphia, Pa. 19118.

Pure 3-sulfopropyl esters, ethers and amides were prepared from fatty acids, alcohols and amides by reaction with 1,3-propanesultone. Their solution properties, including Krafft point, critical micelle concentration, lime soap dispersing power and hydrolytic stability were compared with those of analogous sulfoethyl esters. Detergency was also measured in combination with soap at 150 ppm and 300 ppm water hardness, and compared with soap and a commercially available phosphate built household detergent. The sulfopropyl esters, ethers and amides were more water soluble as shown by their Krafft points which were lower than those of the sulfoethyl esters. The N-3-sulfopropyl amides were better lime soap dispersing agents than the sulfoethyl esters and sulfopropyl esters and ethers. N-3-Sulfopropyl tallowamide was a better detergent in combination with soap, than the other three types of compounds tested, at both water hardnesses, particularly at 150 ppm. The sulfopropyl esters were much more stable to alkaline hydrolysis than the sulfoethyl esters, but only slightly more stable to acid hydrolysis. Sulfopropyl ethers and amides displayed excellent stability to acid and alkaline hydrolysis.

## 18

SOAP-BASED DETERGENT FORMULATIONS. IV: THE SYNTHESIS AND SURFACE ACTIVE PROPERTIES OF SULFOPROPYL ESTERS OF N-SUBSTITUTED IMINODIACETIC ACIDS. W.M. LINFIELD, T.J. MIOICH, M.K. SUCHARSKI and J.K. WEIL, E. Market. Nutr. Res. Div., ARS, USDA, 600 East Mermaid Lane, Philadelphia, Pa. 19118.

A series of fatty amines was converted to N-alkyliminodiacetates with the aid of sodium chloroacetate. N-Acyliminodiacetates were prepared by acylation of dimethyl iminodiacetate with fatty acid chlorides. A series of di(potassium 3-sulfopropyl) N-alkyl and N-acyliminodiacetates was then prepared by reacting 1,3-propanesultone with the above dipotassium N-alkyl and N-acyliminodiacetates, respectively. Examination of surface active properties of the diacetate precursors and their 3-sulfopropyl esters showed good correlation between chemical structure and physical properties. The substituted iminodiacetates showed poor calcium ion stability, lime soap dispersing power and detergency, whereas the 3-sulfopropyl esters of the diacetic acids exhibited high calcium ion stability, good lime soap dispersing power and good detergency. The detergency was enhanced by the addition of soap. The critical micelle concentrations of the 3-sulfopropyl esters were found to be of the expected order of magnitude for dianionic surfactants, and they decreased with increasing molecular weight.

## 19

DIGESTION AND METABOLISM OF RAPESEED OIL BY THE HUMAN AND OTHER SPECIES. BRUCE E. MCDONALD, Dept. of Foods and Nutrition, University of Manitoba, Winnipeg R3T 2N2, Man., Canada.

Two aspects of rapeseed oil (RSO) utilization have been studied in our laboratory: digestion and absorption; and effect of dietary RSO on the fatty acid composition and metabolism of adipose and other tissues. Apparent digestibility of RSO was similar to coconut oil (CO) and lard (La) with young pigs and to CO, La and tallow with growing rabbits. Analysis of digesta from pigs 4 hr postprandial indicated that RSO was more slowly digested than CO and La, although the major portion of digestion and absorption was completed before digesta reached the mid-small intestine. Stearic acid was the major constituent of fecal lipids except for animals fed RSO where erucic or erucic and behenic acids made up 50% of the fecal fatty acids. RSO and erucic acid were well utilized by experimental subjects fed a typical Canadian menu in which 20–22% of the total calories (54% of fat calories) were from RSO and RSO margarine and shortening. Apparent digestibility of erucic acid by adult humans ranged from 98–100%. Inclusion of 10% RSO and tallow in diets of growing pigs did not affect energy utilization, but it had an appreciable effect on fatty acid composition of adipose tissue. Long chain monoenoic fatty acids were readily deposited in adipose tissue of growing pigs, but there was a rapid turnover when RSO was removed from the diet. In states of induced hyperlipogenesis in rats, dietary RSO was as effective as lard and safflower oil in suppressing hyperlipogenesis by liver and adipose tissue. Results in our laboratory indicate that RSO is well utilized and that it is metabolized similar to other dietary fats.

## 20

WHOLESOMENESS OF THE COMPLETE AND PARTIAL ESTERS OF FULLY HYDROGENATED RAPESEED OIL AS DETERMINED IN FEEDING STUDIES WITH RATS. FRED H. MATTSON and G. NOLEN, Procter & Gamble Co., Miami Valley Laboratories, P.O. Box 39175, Cincinnati, Ohio 45239.

Small amounts of rapeseed oil that have been hydrogenated, essentially to a zero iodine value, are used as stabilizers in food systems. A mixture of the mono-, di- and triglyceride of completely hydrogenated rapeseed oil is introduced into other food systems as emulsifiers. These two types of fats were tested for wholesomeness in a 90 day feeding study with rats and for accumulation of lipids in the heart in a 7 day assay with weanling rats. The test fats were fed at several levels, the highest being 18% of the diet. Control groups were fed comparable fats prepared from soybean oil. The growth rate, food consumption, and feed efficiency of the animals fed the fats derived from rapeseed oil and soybean oil were equivalent. Because of their high melting points, the fats from both sources were poorly absorbed. No histological abnormalities were observed in a variety of tissues that were examined. The lipid content of the hearts of the weanling rats was normal.

## 21

BIOCHEMICAL EFFECTS OF DIETARY VERY LONG CHAIN FATTY ACIDS ON RAT HEART AND LIVER. U.M.T. HOUTSMULLER, C.B. STRUYK and A.v.d. BEEK, Unilever Research Vlaardingen/Duiven, Vlaardingen, The Netherlands.

Short-term feeding of oils or fats containing erucic acid to rats induces lipidosis of the heart muscle but not of the liver. Analysis revealed that the fat accumulation consists mainly of an increase in triglycerides containing a high percentage of erucic acid. As carnitine plays an important role in the metabolism of fatty acids by mitochondria and as carnitine deficiency has been found to cause lipidosis, e.g., diphtheria toxin, choline deficiency, the content of carnitine and its esters has been established in heart and liver. In both organs erucic acid caused different changes in these constituents when compared with sunflowerseed oil, indicating that this fatty acid does not induce the usual metabolic responses in these organs. The mitochondria, isolated from hearts of rats fed erucic acid, showed a depressed oxidation rate with various substrates which was proportional to the amount of erucic acid absorbed by the rats. These effects are not specific for erucic acid, but are caused also by its homologues ($C_{20:1}$ $n$-9; $C_{24:1}$ $n$-9) and isomers ($C_{22:1}$ $n$-7 and $C_{22:1}$ $n$-11). A relationship was found between the action of these fatty acids on mitochondria and the chain length or position of the double bond.

## 22

CRAMBE AND RAPESEED OIL CHRONIC TOXICITY STUDY IN RATS. A.N. BOOTH, D.J. ROBBINS, M.R. GUMBMANN, D.H. GOULD, W.H. TALLENT and I.A. WOLFF, W. Reg. Res. Lab. W. Market. Nutr. Res. Div., Berkeley, Calif. 94710.

For 2 years, Fischer rats (25 of each sex) were fed 20% dietary levels of refined, bleached and deodorized soybeans, rapeseed and crambe seed oils, and the same oils after heat treatment to simulate use in deep fat frying. During this time effects on growth, feed consumption and mortality were observed while periodic blood and urine analyses were performed. All surviving rats were autopsied at 2 years; organ weights were recorded, and blood, liver and body fat samples were saved for biochemical analyses. Numerous tissues were preserved in formaldehyde for histopathological examination. The results of this work will be presented, and attempts will be made to correlate the findings with previously reported work on the adverse nutritional effects of high erucic acid-containing oils.

## 23

THE LIPID COMPOSITION OF ERYTHROCYTES AND PLASMA FROM MARINE MAMMALS. GARY J. NELSON, Lawrence Livermore Lab., P.O. Box 808, Livermore, Calif. 94550.

While considerable data have been accumulated on the lipid composition of marine mammals, almost all of these data concern the body fats and oils of commercial interest. Several laboratories have also documented the existence of unusual lipids in specialized organs of marine mammals, such as triglycerides containing isovaleric acid in the melon of marine dolphins. On the other hand, relatively few data are available on the lipids of the blood of marine mammals. This discussion will review briefly the current status of our knowledge in this area and then concentrate on recent data developed in the author's laboratory. Data will be presented on species from the orders Pinnipedia and Cetacea. Red cells and plasma were separated from heparinized, fresh whole blood which was cooled to 0 C immediately after drawing. The lipids were analyzed by thin layer and gas chromatography. The results indicate that the blood lipids of aquatic mammals differ little from those of terrestrial mammals. Unusual lipids in the body organs of aquatic mammals are not found in the circulation of these species. There are, however, some interesting differences between the pinnipeds and cetaceans, in both plasma and red cells. Dolphin plasma also contains variable amounts of an unidentified lipid, believed to be glyceryl ethers, which may be of dietary origin. The fatty acids in the blood of marine mammals generally tend to be more polyunsaturated than those in the blood of terrestrial mammals. Some biochemical implications concerning the metabolism of lipids in marine mammals will be drawn from these data.

## 24

PHOSPHOLIPIDS OF ANTARCTIC SEI WHALE LIVER. FRACTIONATION BY SILVER-NITRATE THIN LAYER CHROMATOGRAPHY. NESTER R. BOTTINO, Texas A&M University, College Station, Tex. 77843.

Phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) were extracted from Sei whale liver, purified and fractionated by silver-nitrate thin layer chromatography. Eight and seven fractions, respectively, were obtained. The fractions were subjected to phospholipase $A_2$ (*Ophiophagus hannah*) hydrolysis to determine fatty acid positional distributions. Sei whale liver contained 18% highly unsaturated fatty acids of 20 and 22 carbon atoms, mostly with five and six double bonds and belonging to the linolenic family (HUFA). PE contained 25% of these acids. In both PC and PE the more unsaturated molecular species contained HUFA esterified to the 2 position and saturated and monoenoic acid (18:0>16:0>18:1>16:1) in the 1 position. Thus the molecular species of whale liver phospholipids have structural characteristics similar to those of land mammals. However, while in land mammals the predominant polyunsaturated molecular species contain tetraenoic acids, in the whale the polyunsaturated species include mainly penta- and hexaenoic fatty acids.

## 25

DISTRIBUTION AND METABOLISM OF ORGANOCHLORINE PESTICIDES AND RELATED COMPOUNDS IN

MARINE MAMMALS. R.F. Addison, Marine Ecology Lab., Bedford Institute of Oceanography, Dartmouth, N.S., Canada.

The distribution of organochlorine pesticides (mainly those of the DDT group and dieldrin) and of polychlorinated biphenyls (PCBs) in some Canadian seals has been studied. Blubber from harp seals from the Gulf of St. Lawrence contained up to ca. 20 ppm ΣDDT (DDT and principal metabolites) and 20 ppm PCB (calculated as Aroclor® 1254). Blubber from ringed seals (for practical purposes a nonmigratory species) from Baffin Island contained about an order of magnitude less ΣDDT, and PCBs were not detectable with any confidence in this group. Levels of ΣDDT and PCB increased consistently with age in the Gulf of St. Lawrence harp seals. The distribution of the principal metabolites DDD and DDE suggested that DDE is the major storage metabolite of DDT in seals, and the low DDD levels suggest that it may be an intermediate in the ultimate degradation of the DDT group compounds.

## 26

CHARACTERIZATION OF UNUSUAL WAX ESTERS FROM THE JAW FAT OF THE ATLANTIC BOTTLE-NOSED DOLPHIN (*Tursiops truncatus*). R. G. Ackman and J. C. Sipos, Fisheries Research Board of Canada, Halifax Lab., P.O. Box 429, Halifax, N.S., Canada, and C. Litchfield and B. Hilaman.

Isovaleroyl esters of long chain fatty alcohols are well documented in the jaw, head and blubber oils of porpoises, dolphins and some other members of the suborder Odontoceti, but this acid is usually the only short chain acid present. An unusual occurrence of isobutyric acid accompanying isovaleric acid in the ratio of 1:3 in the wax esters of the jaw lipids of a specimen of *Tursiops truncatus* is presented, with details of the types of separations obtainable by thin layer chromatography, open-tubular gas liquid chromatography and gas liquid chromatography of unhydrolyzed lipids. The jaw lipid triglycerides contained a much lower proportion of isobutyric acid relative to isovaleric acid 1:20.

## 27

COMPARATIVE LIPID PATTERNS IN THE HEAD FATS OF DOLPHINS, PORPOISES AND TOOTHED WHALES. Carter Litchfield and Anne J. Greenberg, Nelson Biological Lab., Rutgers University, New Brunswick, N.J. 08903.

The unusual isovalerate lipids found in the head fats of the bottle-nosed dolphin have been postulated to play an acoustical role in the echolocation process by which this animal navigates underwater. To determine whether this hypothesis is applicable to other dolphins, porpoises and toothed whales, the head fats of 17 different genera have been examined for lipid class composition and isovaleric acid content using thin layer chromatography and quantitative IR spectroscopy. Animals in the more advanced Delphinidae and Monodontidae families all contain isovaleric acid in their head lipids, while the more primitive Physeteridae, Platanistidae and Ziphiidae families do not. Triglycerides are the major lipid class in all animals examined. Wax esters are present in most species except for the Monodontidae family, the Phocoenidae subfamily of the Delphinidae, and *Platanista gangetica* in the Platanistidae. Where isovaleric acid is present, isovalerate wax esters and diisovalerate triglycerides are the predominant wax ester and triglyceride species found. It is clear, therefore, that no single lipid composition is uniquely correlated with echolocation ability.

## 28

STUDIES OF SEAL AND FETAL WHALE ORGAN LIPIDS. Leon L. Gershbein, Northwest Institute for Medical Research, Chicago, Ill., and R.G. Ackman.

Fresh deep-frozen bodies of a grey seal, *Halichoerus grypus*, a weaned harbor seal, *Phoca vitulina*, and a fetal whale, *Balaenoptera acutorostrata* Lacépède, were thawed and the various organs dissected and extracted with chloroform-methanol 2:1. The processed lipid samples were saponified with alcoholic alkali and the total fatty acids esterified and the methyl esters analyzed by gas chromatography (GC) employing both polar and nonpolar packings. Hydrocarbons were fractionated by chromatography of the unsaponifiable portion over activated alumina and the saturated components further enriched, by column chromatography over silica gel and by use of molecular sieves. The fatty acid composition of such organs as liver, kidney, heart, lung, spleen, brain, muscle, pancreas, thymus, gonad, adrenal and depot fat displayed several variations in the distribution of major acids. The hydrocarbons which constituted up to 55 mg/kg organ except for a two- to three-fold increase in the depot fat, were analyzed by temperature-programed GC employing SE-30 as packing. Up to 32 peaks of $C_{15}$ to $C_{32}$ were observed, and squalene comprised the most prominent unsaturated hydrocarbon. Of great interest, from the standpoint of both turnover and storage, is the marked elevation in pristane in the seal depot fats in contrast to the trace or smaller amounts encountered in the other tissues.

## 29

KINETICAL AND REACTION ENGINEERING ASPECTS OF THE FAT HYROGENATION PROCESS. Niles-Herman Schoon, Chalmers University of Technology, Fack, S-402 20 Göteborg 5, Sweden.

The fat hydrogrenation process is complicated by the presence of slow physical transport steps and very complex chemical steps. The chemical steps include parallel and consecutive reactions involving both *cis-trans* isomerism and double bond migration. The study of the process may be divided into the catalytical factors and the process factors. The catalytical factors include the influence of the catalyst composition, the pore structure and the poisons. The process factors include the reactor construction, the type of process (batch or continuous), the influence of the stirrer rate and the gas flow rate. The rate equations for the hydrogenation of cottonseed oil and rapeseed oil are given, and different types of hydrogenations are simulated and optimized on the basis of these equations. From a study of the mass transfer properties in a pilot scale reactor, the best combination of stirrer rate and gas flow rate for maximum selectivity and maximum content of trans acids is also given.

## 30

KINETICS OF HYDROGENATION OF VEGETABLE OILS. Sava Stefanovic, and Robert H. Price, Dept. of Chemical and Nuclear Engineering, University of Cincinnati, Cincinnati, Ohio 45221, and Lyle F. Albright.

In a continuing effort to further elucidate the mechanism of heterogeneous hydrogenation of vegetable oils, substantially pure methyloleate and methylelaidate as well as mixtures were hydrogenated under conditions of essentially chemical reaction control. This was achieved in a dead-end batch reactor under high rates of agitation but at levels of catalyst concentration, hydrogen pressure and temperature conditions similar to the ones used in industrial plants. Using Nickel catalyst (Nysel from Harshaw Chem. Co.) special attention was directed toward establishing the effect of the process variables on rates of isomerization and concurrent hydrogenation of the geometrical and positional isomers. The results obtained are compared with comparable results of hydrogenation of cottonseed oil. The conclusions drawn will be of considerable significance to the understanding of the mechanism of hydrogenation of vegetable oils.

## 31

DEUTERATION OF METHYL LINOLEATE WITH NICKEL, PALLADIUM, PLATINUM AND COPPER-CHROMITE CATALYSTS. Sambasivarao Koritala, E. Selke and H.J. Dutton, N. Reg. Res. Lab., 1815 N. University St., Peoria, Ill. 61604.

Methyl linoleate was reduced with the title catalysts in the presence of deuterium. Samples taken during reduction were separated into saturate, monoene and diene fractions. To calculate the amount of linoleate that was reduced through a conjugated intermediate, profiles for the isomeric monoenes were compared with those from hydrogenation of alkali-conjugated linoleate. Up to 57% of the linoleate was reduced through a conjugated intermediate with nickel catalyst. The respective percentages for palladium and platinum catalysts were 51 and 25. Copper catalysts have previously been shown to reduce linoleate through conjugated intermediates. The copper-chromite catalyst showed absolute selectivity for the reduction of linoleate to monoene since stearate did not form. Nickel exhibited the next highest selectivity; palladium, moderate selectivity; platinum, none. Computer simulation of linoleate reduction with platinum indicated that ca. 20% of the linoleate was directly reduced to stearate through a shunt. Geometrical isomers of linoleate were formed during reduction with all catalysts except copper-chromite. The nickel catalyst formed both *trans,trans-* and *cis, trans-*isomers, as well as nonconjugatable dienes. Deuterium was incoporated into these isomers. Because conjugated dienes are more reactive than linoleate, they were not observed during reduction with nickel and palladium catalysts. Copper-chromite catalyst isomerized linoleate to conjugated dienes only. Deuterium was found in these conjugated dienes, which were also extensively isomerized. As a result of isomerization and exchange during reduction of linoleate, as well as further exchange between deuterium and monoenes, a wide distribution of isotopic isomers in monoenes was observed with nickel, palladium and platinum catalysts. Since isomerization of monoenes with copper-chromite is negligible, the isotopic distribution of monoenes must be due to isomerization of the intermediate conjugated dienes followed by addition.

## 32

STOICHIOMETRY OF COTTONSEED EXTRACTION. William H. King, S. Market. Nutr. Res. Div., P.O. Box 19687, New Orleans, La. 70179.

Cottonseed, with its importance as a vegetable oil source for human food and its potential as a major source of high quality protein, has been studied extensively in recent years in an effort to develop processes for its extraction to separate desired components from biologically inactive or undesired elements of its composition. Because of its complexity and the delicate nature of its protein, processes for separating the useful from the nutritionally undesired portions are complicated. This paper presents results of a study of methods to measure the components of interest at different stages of an experimental extraction process. Such measurement is essential to intelligent design of processing procedures to achieve the desired separations. While the stoichiometric outline presented is based on a mixed solvent experimental extraction of raw cottonseed flaked meats, the principles involved should be applicable to other complex experimental separation procedures. Specific data are presented for analysis of the raw flakes, marcs, miscellae, extracted flakes, and oils obtained in a 10 step extraction procedure together with the mathematics employed in calculating the composition of the various intermediate products from the conventional analytical data obtained.

## 33

WATER-RECYCLE WASHING OF REFINED SOYBEAN OIL: PLANT-SCALE EVALUATION. R. E. Beal, L. T. Black and E. L. Griffin, N. Reg. Res. Lab., 1815 N. University St., Peoria, Ill. 61604, and J. C. Meng and G. S. Farmer.

A series of 24 hr tests was made in a commercial refinery under eight different operating conditions to select optimum specifications for a subsequent longer test of the recycle-washing process. Alkali-refined oil was continuously washed for sodium removal at a rate of 15,000 lb/hr. Wash water was reused without discard. Wash-water pH levels of 2.5 and 3.0, oil-water ratios of 2 and 5, and the addition of ethylene diamine tetraacetic acid (EDTA) to wash water were factors investigated. Samples of oil and water taken during the tests were analyzed for sodium, iron and copper by atomic absorption, and laboratory hydrogenation tests were made on the oils. For the longer test a water pH of 3.0 and an oil-water ratio of 4:1 were used and EDTA was not added to the recycle water. Operating and analytical data, equipment specifications and cost data were acquired. Certain problems encountered during operation were solved.

## 34

THE TESTING OF COMMERCIAL HYDROGENATION CATALYSTS. R. R. Allen and J. E. Covey, Anderson Clayton Foods, P.O. Box 63, Richardson, Tex. 75080.

To approve a hydrogenation catalyst for use in a manufacturing plant, the catalyst should be tested for its activity, selectivity, isomerization activity and filterability. By use of the catalyst to hydrogenate soybean oil under controlled conditions the activity, selectivity and isomerization activity may be determined. To de-

termine the filterability, the catalyst is suspended in $CCl_4$ and filtered through a 0.75$\mu$ millipore filter. The absorbance of the filtrate is a measure of the filterability. The results of the tests are compared to standard values and the catalyst approved or rejected.

## 35

FATTY WASTE PRODUCT UTILIZATION IN THE FOOD INDUSTRY. L. EMARD, Laval University, Queb., Canada.

Abstract not available at press time.

## 36

A WATER POLLUTION CONTROL PROGRAM TYPICAL OF THE MEAT PACKING INDUSTRY. G. H. KESTLE and G. S. MOSS, J. M. Schneider Ltd., 321 Courtland Ave. E., Kitchener, Ont., Canada.

The water effluent from this particular industry is similar to domestic sewage in that it is biodegradable, but much higher in strength. Waste treatment includes preliminary fat recovery in the packing house, the separation of fat-bearing wastes from manure-bearing wastes, and the treatment of these two effluents by a separate primary system. The common effluent which is discharged to the municipal sewage treatment system is reduced in strength by these operations to a value acceptable to the present effluent strength limits. In anticipation of lower effluent strength limits in the future, an investigation is being carried out in cooperation with the Water Research Institute of the University of Waterloo to determine the treatability of this type of waste with a plastic media, trickling filter, secondary system. Continuous monitoring is important to an effective water pollution abatement program.

## 37

REMOVAL AND RECOVERY OF FATTY MATERIALS FROM EDIBLE FAT AND OIL REFINERY EFFLUENTS. W. C. SENG, Swift & Co. R&D Center, 1919 Swift Drive, Oak Brook, Ill. 60521.

A 2 year study in cooperation with the FWQA has been conducted to define and treat effluent from one of Swift's edible oil refineries. As a result of these studies, the existing treatment system was modified to produce effluent suitable for discharge and at the same time eliminate surcharges and the need for a lagoon. The treatment system consists of a skim tank which is cathodically protected, automatic pH and chemical addition control and an air flotation cell also cathodically protected. Of particular significance is the inedible grease recovery system. The skimmings from the skim tank and from the air flotation cell are acid-treated, heated and centrifuged to recover 7–10,000 lb./day of oil. Sale of recovered inedible grease can cover 60-80% of the direct costs of operation of the waste treatment system. Recent improvements in the system have reduced even further the cost of treatment and raised effluent quality. With impressed current and chemicals the BOD is reduced from 3300 to 150, FOG from 2500 to 75, and Suspended Solids from 3000 to 150 ppm.

## 38

ADVANCED PROCESS SYSTEMS FOR THE CONSERVATION, RECLAMATION AND REUSE OF SOLIDS AND WATER—THE SOLUTION TO POLLUTION. R.A. GALLOP, Dept. of Food Science, University of Manitoba, Winnipeg, Man. R3T 2N2, Canada.

Environmental pollution is a measure of our inefficiency in materials management. Every waste generating process produces a raw material for our potential use. We must minimize the formation of such wastes and productively use them, preferably by recycling, where feasible, or render them innocuous to the environment before discharge. Water rather than being "consumable" is almost wholly used as a cylic transfer fluid, in nature and throughout human society. We must build fixed and mobile, accelerated physico-chemical refining systems for several classes of process water intended for reuse, rather than for discharge. Then we can reduce the environmental problems which might arise from our water and wastes, to minor levels, in many cases much lower than the levels which occur naturally. In doing so we can magnify our useful resources tremendously, so that we can supply the resource needs of anticipated populations by providing a much cleaner environment, while using less of our reserve resources per capita than at present, plus getting rid of most of our garbage dumps and attendant problems. To do all this complex janitorial work, we will generate the most beneficial, largest, cleanest industrial growth ever seen. This paper will review the principles and practices of good materials management in process, with particular reference to edible oils processing. It will be illustrated by examples from research, pilot plant and industrial practices throughout the world. Work of this type has been under way at the Dept. of Food Science, University of Manitoba, for several years, and latest integrated waste management systems will be discussed.

## 39

EFFECTS OF DIETARY POLYUNSATURATED FATTY ACIDS UPON TAIL DEVELOPMENT AND LIVER LIPID COMPOSITION OF IMMATURE NORMAL AND HYPOPHYSECTOMIZED RATS. O. S. PRIVETT, E. W. HAEFFNER, The Hormel Institute, 801 16th Ave. N.E., Austin, Minn. 55912.

Studies of the effect of dietary polyunsaturated fatty acids on the development of scaly tail symptoms similar to an EFA deficiency using hypophysectomized immature rats of the Sprague-Dawley strain are reported. Hypophysectomized weanling rats fed a semipurified fat-free diet supplement with 10% of either corn oil, hydrogenated coconut oil, an arachidonate concentrate (40–50%, 20:4), menhaden oil concentrate (20%, 20:5) or linseed oil for ca. 8-12 weeks were killed and their livers used for fatty acid and lipid class analysis. The data obtained were compared to those of normal animals which were fed the same diets. The hypophysectomized rats, especially those fed the polyunsaturated fatty acid concentrates and the hydrogenated coconut oil, exhibited scaly tails and feet characteristic of the dermal symptoms of an essential fatty acid (EFA) deficiency. The caudal necrosis of the arachidonate-fed rats often developed to such an extent that part of the tails dropped off after ca. 8 weeks. The fatty acid composition of the liver of the hypophysectomized rats showed that the interconversion between the various fatty acid species was not impaired as compared to the normal animals. However livers of the hypophysectomized rats contained small but significantly higher concentrations of eicosatrienoic acid than normal animals. The lipid class analysis showed that the percentage of cholesterol and cholesterol esters in the liver increased and that of the phospholipids decreased in the hypophysectomized animals.

## 40

NATURE OF STEROLS AND STERYL ESTERS IN AORTA AND PLASMA OF ATHEROSCLEROSIS-SUSCEPTIBLE WHITE CARNEAU PIGEONS ON CHOLESTEROL-FREE DIETS. M. T. RAVI SUBBIAH, Mayo Clinic, Rochester, Minn. 55901.

The nature of specific sterols in the free and esterified sterol fractions and the component fatty acids from the aorta and plasma of White Carneau pigeons (a breed susceptible to spontaneous atherosclerosis) on cholesterol-free diets were examined using thin layer chromatography (TLC), argentation TLC, and gas liquid chromatography. In the aortic tissue, the mean concentration of total sterol was 1.87 mg/gm wet tissue, of which 21.9% was esterified. Cholesterol contributed 92–94% of the total sterols in both the free and the esterified sterol fraction with cholestanol (2-5%), desmosterol and an unidentified diene sterol (<1%). In the plasma, 72.5% of the sterols was present as esters. The sterol pattern of the plasma showed a similar distribution with cholestanol contributing 2.4% of the total sterols. These pigeons also excreted a large amount of cholestanol (20% of the total sterols in the cholesterol group in the feces), suggesting that this stanol could play a role in the development of atherosclerosis in this species. The major fatty acid in the aortic steryl ester fraction was oleic acid (46.1%); others were palmitic (24.5%), stearic (12.8%), linoleate (12.3%) and palmitoleic (4.5%). However, in the plasma of these pigeons, linoleate was the major fatty acid in the steryl ester fraction (48.6%); oleic (28.0%), palmitic (10.4%), palmitoleic (4.8%), stearic (3.9%), arachidonic (2.0%), and unidentified (2.3%) fatty acids made up the rest. This preferential presence of oleic acid in the steryl ester fraction of the pigeon aorta is similar to that of the diseased human artery. The differences in the fatty acid composition between the aortic tissue and plasma of these pigeons suggest that the bulk of the aortic steryl ester fatty acid could be of endogenous origin in this species.

## 41

DIETARY FACTORS AND AFLATOXIN TOXICITY. ROSLYN B. ALFIN-SLATER, LILLA AFTERGOOD, and PENNY WELLS, School of Public Health, University of California, Los Angeles, Calif. 90024, and DANIEL MELNICK.

Studies in this laboratory involving interrelationships between diet and aflatoxin toxicity in rats have indicated that the tumorogenic and biochemical response to the toxin is affected by the diet. In rats fed diets containing either peanut oil and a varied natural protein source (Diet I); or lard, casein as the sole protein and a less complete vitamin mixture (Diet II), differences were observed in intensity of tumor development and in biochemical responses. This present study was initiated to clarify the possible importance of the fat source as opposed to some other component of the diet in altering these biochemical responses. For this purpose a crossover experiment was devised in which the two fats to be studied were reversed in the two diets containing different protein sources and vitamin mixtures while other components were kept constant. It was found that plasma cholesterol levels increased significantly in response to aflatoxin, more so, however, when lard was fed with the casein as protein source. It was also found that the fatty acid patterns in the sterol ester fraction of plasma and in all the lipid fractions of liver of rats fed lard were suggestive of EFA deficiency as compared to those fed peanut oil; this condition was markedly more pronounced when lard was fed with Diet II than when lard was fed with Diet I. Pathological examination of the livers indicates that tumor formation is more extensive in animals fed Diet II as compared to Diet I. In addition it was observed that when lard was substituted for peanut oil in basal Diet I, the severity and incidence of tumors did not change significantly. However, when peanut oil was substituted for lard in basal Diet II, the incidence, but not the severity, of the tumors was decreased. These findings suggest that some component of basal Diet II other than the fat source alone is responsible for the increased pathology observed among these animals and also for the decreased levels of the essential fatty acids found in tissues.

## 42

GLYCERIDE METABOLISM IN ISOLATED MUCOSAL CELLS. P. J. A. O'DOHERTY, I. M. YOUSEF and A. KUKSIS, Banting and Best Dept. of Medical Research, 112 College Street, Toronto 101, Ont., Canada.

Previous studies from our laboratory have led to the isolation of fat-laden microvillous cells from rat intestinal mucosa and the demonstration that the release of chylomicrons from these cells is dependent on both protein and phospholipid biosynthesis.

We now report that the isolated cells are capable of effective triglyceride synthesis and an apparently normal formation of chylomicrons when incubated in bicarbonate buffer. For this purpose, microvillous cells isolated from fasted rats were used along with cells from which the chylomicrons had already been released once in vitro. The fat-depleted cells absorbed monoglycerides and free fatty acids added as bile salt micelles at rates comparable to those seen in evert sacs and became laden with fat permitting their reisolation by flotation. The presence of prechylomicrons in these cells was confirmed by electron microscopy. Upon addition of albumin, the cells released the newly synthesized triglycerides as chylomicrons, which were isolated by Millipore filtration. Incubation with labeled 2-monoolein gave rise to a minimum of 33-37% 2,3-diglycerides and a maximum of 63-67% 1,2-diglycerides. It was concluded that triglyceride biosynthesis from 2-monoglycerides in isolated cells proceeded via both 1,2- and 2,3-diglyceride intermediates, as already claimed for evert sacs, and unlike the synthesis in isolated microsomes which occurs exclusively via 1,2-diglycerides.

## 43

GLYCOLIPIDS OF PERIPHERAL NERVE: ISOLATION AND CHARACTERIZATION OF GLYCOLIPIDS FROM RABBIT SCIATIC NERVE. HARBHAJAN SINGH, Dept. of Medicine,

New York University School of Medicine, and Lipid Metabolism Lab., Veterans Administration Hospital, New York, N.Y. 10010.

Besides cerebroside and sulfatide, four other glycolipids were isolated from rabbit sciatic nerve and analyzed by chemical and chromatographic methods. Three of them were shown to be fatty acid esters of cerebroside. The fourth glycolipid was characterized as diacyl glycerol galactoside and its alkyl ether analog. In the ester linkage mainly short chain unsubstituted acids with chain length C-16 to C-18 were present. Both hydroxy and unsubstituted acids were present in amide linkage. They varied in chain length from C-16 to C-24 and were typical of cerebrosides. The long chain base fraction contained sphingosine and dihydrosphingosine as the main components. How the three cerebroside esters described above differ from each other will also be discussed.

## 44

SYNTHETIC TRIGLYCERIDES AS STEREOSPECIFIC PROBES IN ENZYMIC LIPOLYSIS. N. Morley, A. Kuksis and D. Buchnea, Banting and Best Dept. of Medical Research, 112 College Street, Toronto 101, Ont., Canada.

*sn*-Glycerol-1-palmitate-2-oleate-3-linoleate and its enantiomer were prepared by chemical synthesis from D-mannitol and the appropriate acyl chlorides. The triglycerides were subjected to enzymic hydrolysis in appropriate buffers following initial emulsification with glycocholic acid and activation with cofactors, if necessary. The 1,2- and 2,3-diacyl-*sn*-glycerols released as intermediates were quantitated by gas chromatography as the trimethylsilyl ethers and by argentation thin layer chromatography as the acetates-1-$C^{14}$. Alternatively, analyses were made with synthetic triglycerides containing radioactivity in the glycerol or oleic acid moieties of the enantiomers. While pancreatic lipase showed a slight preference for the formation of 1,2-diglycerides, lipoprotein lipase gave a marked preferential release of the 2,3-diglycerides. The above results are similar to those obtained with radioactive triolein and conventional stereospecific analysis. In contrast to conventional analyses, these assays are more rapid and much more direct, and therefore less likely subject to error due to involved analytical manipulation. The stereochemical course of the above hydrolyses may also be assessed by the release of the characteristic fatty acids from the 1 and 3 positions, which may be monitored by their mass, radioactivity or the uptake of $H_2O^{18}$ under appropriate incubation conditions and with suitable instrumentation.

## 45

PALMITYL CoA REDUCTASE, PALMITALDEHYDE REDUCTASE AND PLASMALOGEN BIOSYNTHESIS IN *CLOSTRIDIUM BUTYRICUM*. Per-Otto Hagen and Lizzie Barber, Duke University Medical Center, Dept. of Surgery, Durham, N.C. 27710.

Dialyzed cell free preparations of *C. butyricum* reduce palmityl CoA to cetyl alcohol in the presence of NADH and NADPH. The enzymes are localized in the 100,000 × g supernatant fraction after EDTA plus lysozyme treatment of logarithmically growing cells. Palmityl CoA reductase and palmitaldehyde reductase have been separated by Sephadex G-100 gel filtration chromatography. The reduction of palmityl CoA to palmitaldehyde is reversible and requires NADH. NADPH can partially substitute as cofactor. The reduction of palmitaldehyde to cetyl alcohol is only slightly reversible and requires NADPH. The synthesis of alk-1'-enyl glyceryl ethers (plasmalogens) in *C. butyricum* has been studied by growing cells in the presence of 1-$^{14}$C-1-$^{3}$H-cetyl alcohol and in pulse-chase experiments using $^{32}$P-orthophosphate and glycerol-2-$^{14}$C as polar lipid precursors. About one-half of the tritium from the doubly labeled cetyl alcohol is recovered in the vinyl ether linked chain of the plasmalogens, suggesting the involvement of long chain alcohols or their oxidation product aldehydes in plasmalogen synthesis. Pulse-chase experiments, using $^{32}$P-orthophosphate as polar lipid precursor, show a reciprocal redistribution of radioactivity from the diacylphosphatidylethanolamine into the 1-alk-1'-enyl-2-acyl glycerylphosphoryl-N-methylethanolamine. No reciprocal redistribution of radioactivity is seen when glycerol-2-$^{14}$C is used as polar lipid precursor in pulse-chase experiments. Based on these observations, it is concluded that diacylphosphatides are the precursors for plasmalogen biosynthesis in this organism, through a mechanism of phosphoryl-base transfer.

## 46

DISTRIBUTION OF L-SERINE-$C^{14}$ AMONG MOLECULAR SPECIES OF GLYCEROPHOSPHOLIPIDS. S.K.F. Yeung and A. Kuksis, Banting and Best Dept. of Medical Research, 112 College Street, Toronto 101, Ont., Canada.

The incorporation of L-serine-U-$C^{14}$ and L-serine-3-$C^{14}$ into various species of rat liver phosphatides as a function of time was determined in vivo following iv injection. The purified phosphatides were resolved according to degree of unsaturation by argentation thin layer chromatography. The phosphatidylethanolamines (PE) and phosphatidylserines (PS) were chromatographed as the trifluoroacetamides while the phosphatidylcholines (PC) were run in the native form. There was a rapid initial labeling of PC which reached a maximum at 5 hr, after which it gradually declined. PS showed a rapid initial labeling of a lower magnitude than PC. PE became labeled only gradually, but surpassed PS after the 5th hr and continued to rise to the 9th hr. At 15 min, the relative specific activity (specific activity of species/specific activity of lipid class) of all species of PS were similar, and about three times lower than that of the hexaenoic PC and two times lower than that of oligoenoic PE. Most other species of PC and PE possessed relative specific activity which were significantly lower than those of PS. With progressing time, the relative specific activity of polyenoic PS changed little while those of the oligoenoic PS declined. Also, relative specific activity of hexaenoic PC decreased; that of the tetraenoic PC slightly increased, while that of the oligoenoic PC remained unchanged. The relative specific activity of oligoenoic and hexaenoic PE declined with time, and that of the tetraenoic PE increased. The above results are accounted for by postulating a rapid generation of labeled methyl groups from L-serine-$C^{14}$, which are used for preferential conversion of hexaenoic PE to PC; an exchange of L-serine for another nitrogenous base in glycerophospholipids; and a preferential decarboxylation of oligoenoic PS to PE, along with a general interconversion of oligoenoic into tetraenoic species of PC, PE and possibly PS.

## 47

LIPIDS AND FATTY ACIDS OF THE SOUTHWESTERN CORN BORER, *Diatraea grandiosella*. A.C. Thompson, Frank M. Davis, R.D. Henson, R.C. Gueldner, P.A. Hedin and C.A. Henderson, Entomology Res. Div., Boll Weevil Res. Lab., P.O. Box 5367, State College, Miss. 39762.

The lipids of adults and several immature stages of the southwestern corn borer, *Diatraea grandiosella*, fed natural and artificial diets were studied. The fatty acid contents of the neutral and phospholipids from natural and artificial diet are discussed. The relationships of the phospholipids are discussed in terms of their saturated and unsaturated fatty acid content. Fat body lipids and fatty acids from fat bodies of diapausing larvae from natural and synthetic diets are compared along with the lipid content of the hemolymph.

## 48

THE EFFECT OF ENVIRONMENT AND GENETICS ON ERUCIC ACID IN COMMERCIAL CANADA RAPESEED. B. Craig, D. Irvine, Whyte, J.R. Reynolds and K. Downey, Canada Dept. of Agriculture, Saskatoon, Sask., Canada.

Abstract not available at press time.

## 49

SELECTION FOR LINOLEIC AND LINOLENIC ACID IN RAPESEED. Gerhard Rakow, Res. Station, Res. Branch, Canada Agriculture, University Campus, Saskatoon, Sask., Canada.

An increase in linoleic and a decrease in linolenic acid content in the seeds of rape plants is one of the most important points of interest for the plant breeder. In the progeny of a mutation experiment with seeds of the variety "Oro" two mutants were selected, one with 5% and the other with 20% linolenic acid (normal = 8–10%). The linoleic acid content of the two mutants was the same as the normal "Oro-Type" (16–20%), but the oleic and linolenic acids were inversely related. Environmental conditions particularly influenced the content of linolenic acid in a very wide range. Crossing experiments indicated that the level of linolenic acid was largely determined by the genotype of the developing embryo. Maternal effects were also observed. Results indicated that in the desaturation-chain 18:1 → 18:2 → 18:3 two different enzymatic reaction steps exist, one regulating the transformation from 18:1 to 18:2, the other the transformation from 18:2 to 18:3. Two different enzymatic reaction steps are an indispensable requirement for desired breeding work.

## 50

PLACE OF RAPESEED IN THE EDIBLE OIL MARKET. James McAnsh, Rapeseed Association of Canada, 1015, 837 West Hastings Street, Vancouver 1, B.C., Canada.

To obtain some measurement of the potential for rapeseed oil in the world edible oil market, one need look no further than the statistics of net exports of the principal vegetable **oilseeds from primary producing countries**. The last complete year for which figures are available is 1970, and in that year, according to the Commonwealth Secretariat in London, England, soybeans account for ca. 52% and rapeseed only ca. 7.5% in oil equivalent. Since soybeans have only ca. 0.5 the oil content of rapeseed they are bought mainly for their yield of high protein meal. Conversely rapeseed is bought for its oil content and produces a meal that is not only lower in protein but up to this time has been less acceptable as an ingredient in animal feed formulations. Fortunately for rapeseed the problems are being tackled diligently and hopefully will be overcome in the near future. When this point has been reached, rapeseed will be a much stronger competitor in world markets for protein meal. The trend in the use of rapeseed oil in the Canadian domestic market is an indicator of the potential in world markets. It is definitely displacing other edible oils that have dominated our home market in the past and in 1971 calendar year. The 160.5 million pounds used in the manufacture of margarine, shortening and salad oils represented 35.6% of the total vegetable oils used in these products. Rapeseed oil is competing keenly with soybean oil in the domestic market and should be able in the future to greatly enlarge its share of world trade.

## 51

THE LIPID BIOCHEMISTRY AND ULTRASTRUCTURE OF DEVELOPED RAPESEED. L.-A. Appelqvist, University of Lund, Sweden.

Abstract not available at press time.

## 52

COMPARISON OF CHEMICAL AND AGRONIMIC CHARACTERISTICS OF THE SPRING RAPE (*Brassica napus*) VARIETIES BRONOWSKI AND TARGET. A.J. Finlayson, J. Krzymanski and R.K. Downey.

Abstract not available at press time.

## 53

VARIETAL DIFFERENCES IN PROTEIN OF ORIENTAL MUSTARD (*B. juncea L.*). S.L. MacKenzie.

Abstract not available at press time.

## 54

BIOSYNTHESIS OF LIPIDS IN THE MARINE COPEPOD, *Euchaeta norvegica*. J.R. Sargent, R.R. Gatten and R. McIntosh, Institute of Marine Biochemistry, St. Fittick's Road, Aberdeen, Scotland, AB1 3RA.

The 1000 g supernatant of a homogenate of whole, adult *Euchaeta* (an animal rich in wax esters) incorporates either ($^{14}$C) palmitic acid in the presence of ATP, coenzyme A and magnesium ions, or ($^{14}$C) palmityl coenzyme A into phospholipids, triacylglycerols and wax esters. Incorporation into wax esters is 5–10 times incorporation into triacylglycerols. Incorporation of ($^{14}$C) palmitic acid into wax esters is markedly dependent on the concentration of added oleyl alcohol, 10 times as much radioactivity being incorporated at the optimal concentration (0.3 mM) as in the absence of added oleyl alcohol. Above 0.3 mM oleyl alcohol inhibits incorporation of ($^{14}$C) palmitic acid into wax esters. In the presence of added

NADPH, ATP and coenzyme A, or palmityl coenzyme A, palmitic acid is converted to palmityl alcohol, and the synthesis of wax esters is stimulated. A whole cell preparation of *Euchaeta* will incorporate radioactivity from a variety of precursors into wax esters and triacylglycerols, the former lipid being more heavily labeled than the latter. These precursors include (U-$^{14}$C) glucose, ($^{14}$C) pyruvate, ($^{14}$C) acetate and ($^{14}$C) alanine. Radioactivity from (U-$^{14}$C) glucose is incorporated into both the acid and alcohol moieties of wax esters. Both L-malate: NAD and L-malate:NADP oxidoreductase activities are present in *Euchaeta*. The results are consistent with the concept that conversion of fatty acid to fatty alcohol is a rate-determining step in biosynthesis of wax esters. The extent to which de novo biosyntheses of both fatty acids and fatty alcohols are involved in controlling the level of wax esters, and the relationship of such biosyntheses to the redox potential of the cell will be discussed.

## 55

A UNIQUE LYSOPHOSPHOLIPID IN MARINE COPEPODS. RICHARD F. LEE, STUART PATTON and A.A. Benson, Scripps Institution of Oceanography, La Jolla, Calif. 92037.

Copepods, which account for a major part of the zooplankton in the ocean, are well known for their ability to store lipids. Our previous work showed that wax esters are the main storage lipid of many copepods. Investigation of the North Pacific copepod, *Calanus plumchrus*, revealed unusual concentrations of lysophosphatidyl ethanolamine (48% of the phospholipid) in animals actively grazing a spring diatom bloom. This lysophospholipid was composed largely of docosahexaenoic acid (78% 22:6). Phosphatidyl choline, sphingomyelin, phosphatidyl ethanolamine, lysophosphatidyl choline accounted for 17%, 13%, 11% and 6% of the phospholipid, respectively. All of these phospholipids were highly unsaturated with a predominance of docosahexaenoic acid (between 43% and 54%). Analysis of *C. plumchrus* collected during January, when phytoplankton levels are low and wax ester synthesis was not occurring showed only traces of lysophosphatidyl ethanolamine. Thus we speculate that lysophosphatidyl ethanolamine may be involved in the transport of fatty acids utilized in the synthesis of wax esters. A survey of copepods which do not synthesize wax esters showed the principal phospholipids to be phosphatidyl choline, phosphatidyl ethanolamine, and sphingomyelin.

## 56

LIPIDS OF MARINE CRUSTACEANS. JUDD C. NEVENZEL and NIRMALA K. MENON, Lab. of Nuclear Medicine and Radiation Biology, University of California, 900 Veteran Avenue, Los Angeles, Calif. 90024.

Among the gustatory delights from the oceans are the large crustaceans: prawns, shrimps, crabs and lobsters. The portions most enjoyed are the muscle tissues, which are low in total lipid (2–5% of dry wt) and relatively high in both sterol (10–30% of total lipid) and phospholipid (30–60%). We report the analyses of the lipids from a penaeid prawn and the blue crab, *Callinectes sapidus*. The former contains only 7% neutral energy reserves; of this, 1.9% is wax ester, largely hexadecyl oleate. Phosphatidyl choline constitutes 10% of the total lipid in the prawn, phosphatidyl ethanolamine, 4.5% and phosphatidyl serine plus phosphatidyl inositol, together ca. 7.5%. The fatty acid patterns of the triglyceride, free fatty acids, PC, PE, PS and PI fractions were determined, that of the PI was noteworthy in containing less than 7% total polyunsaturated acids. We examined the free sterol fractions by gas liquid chromatography, searching specifically for desmosterol. In two preparations from the penaeid prawns and in the crab, lipid desmosterol was tentatively identified, constituting ca. 1% of the total sterol; in all cases cholesterol was 98+%.

## 57

SPHINGOLIPIDS IN MARINE SHELLFISH. AKIRA HAYASHI, TOSHIKO MATSUBARA and FUMITO MATSUURA, Dept. of Chemistry, Faculty of Science and Technology, Kinki University, Higashiosaka, Osaka, Japan.

The occurrence of sphingophosphonolipids (SPL's) has been reported in shellfish and sea anemone, and two types of SPL, i.e., ceramide 2-aminoethylphosphonate and ceramide 2-N-methylaminoethylphosphonate were well known. However detailed analyses of fatty acids, long chain bases (LCB's) and ceramides have not been reported. SPL fraction was prepared from seven kinds of marine shellfish (*Monodonta labio, Turbo cornutus, Cellana eucosmia, Conomurex luhanus, Ostrea gigas, Mytilus edulis* and *Liolophura japonica*), and fatty acids, LCB's, and ceramides of the SPL's were analyzed by gas chromatography-mass spectometry (GC-MS). In all shellfish, the main fatty acid was palmitic acid ($C_{16:0}$) (>80%) except in *C. eucosmia* and *L. japonica* which contained considerable amounts of 2-hydroxy palmitic acid ($C_{16:h}$). The contents of $C_{16:0}$ and $C_{16:h}$ were 65% and 15%, respectively in *C. eucosmia*, and 50% and 30%, respectively, in *L. japonica*. The LCB fraction showed a complex pattern, characteristics for each species of shellfish. Dienic LCB was predominant in all shell fish SPL's i.e., $C_{18}$-sphingadienine (*d*18:2) in *O. gigas* (55.7%); $C_{20}$-sphingadienine (*d*20:2) in *C. eucosmia* (50.8%) and in *L. japonica* (25.1%); and $C_{22}$-sphingadienine (d22:2) in *T. cornutus* (86.3%). Furthermore the intact SPL were trimethylsilylated (TMS) and analyzed directly by GC-MS without prior degradation. TMS derivatives of the SPL were separated according to ceramide moieties on GC, and the molecular species of ceramide moieties were identified by their characteristic LCB and ceramide ions on MS. Thus it was found that *d*18:2 in *O. gigas* and *d*22:2 in *T. cornutus* were combined to $C_{16:0}$, but *d*20:2 was combined to $C_{16:0}$ and $C_{16:h}$ in *C. eucosmia* and *L. japonica*. The ratio of the combination *d*20:2-$C_{16:0}$ to *d*20:0-$C_{16:h}$ was ca. 1:1 in *C. eucosmia* and 1:4 in *L. japonica*.

## 58

PHOSPHOSPINGOLIPIDS OF SHELLFISH. TARO HORI, OSAMU ITASAKA and MASAO IWAMORI, Dept. of Chemistry, Shiga University, Ohtsu, Shiga, Japan, and Mutsumi Sugita.

After the finding of ceramide aminoethylphosphonate (CAEP), ceramide N-methylaminoethylphosphonate (CMAEP) and ceramide phosphorylethanolamine (sphingoethanolamine; CPEA) from the shellfish, the presence of approximate amounts of N-acyl CAEP and N-acyl CMAEP has been presumed in our laboratory. In the present studies these new lipids were semi-synthesized using CAEP and CMAEP from bivalves, respectively. In the physical properties including NMR, IR and $[\alpha]_D$, and in the elemental analysis, there was not a great difference between the synthesized and natural compound. On the other hand, CPEA that has not been isolated from the bivalves was detected in *Corbicula sandai* and *Pinctada martensi*. We are interested in the following facts that no trace of $C_{18}$-sphingosine was gas chromatographically detected but a number of long chain bases shorter than $C_{18}$ were found when based on relative retention times of their trimethylsilyl derivatives.

## 59

STUDIES ON THE DIGESTIVE LIPASE OF THE SURF CLAM, *Spisula solidissima*. JOHN S. PATTON and JAMES G. QUINN, Graduate School of Oceanography, University of Rhode Island, Kingston, R.I. 02881.

Crude lipase was prepared by lyophilizing the crystalline styles of *Spisula solidissima*. The crude lipase remained stable under refrigeration and showed a specific activity <1% of hog pancreatic lipase. Basic studies showed the clam lipase to have pH and temperature optimums of 8.0 and 18 C, respectively. A series of experiments was run under these conditions to compare the clam lipase activity with the standard hog pancreatic lipase on a variety of substrates. The clam lipase was found to be the more versatile enzyme on wax esters as well as methyl esters. Neither of the two lipases was capable of hydrolyzing the secondary alcoholic esters of glycerol, cholesterol or isopropyl alcohol; however both showed activity on the primary alcohol, isobutyl oleate. *S. solidissima* lipase, hog pancreatic lipase, as well as an additional lipase isolated from the pancrease of the little skate, *Raja erinacea*, all demonstrated an *inability* to hydrolyze the methyl esters of arachidonic, eicosapentaenoic and docosahexaenoic acids. The relationship of this work to the study of marine fat structures is discussed. A consideration of the effects of lipophilic pollutants on marine lipases is also included.

## 60

COMPARATIVE BIOCHEMISTRY OF JELLYFISH: FATTY ACIDS OF SCYPHOZOAN POLYPS AND MEDUSAE AND THE EFFECT OF DIET ON FATTY ACID COMPOSITION. JEANNE D. JOSEPH and EDWIN B. JOSEPH, S.C. Marine Research Lab., P.O. Box 12559, Charleston, S.C. 29412, and Paul L. Zubkoff.

In characterizing the biochemical processes associated with the polymorphs of Scyphozoan jellyfishes, the lipids provide several classes of "small" but complex molecules which may possibly serve as temporal markers in their development or tracers for food chain studies. Fatty acids (FA) of the CHCl$_3$/CH$_3$OH extracts of *Chrysaora quinquecirrha* polyps and medusae were quantified with gas liquid chromatography (SE-30, DEGS, EGSS-X) after transesterification with BF$_3$/CH$_3$OH. The percentage FA of laboratory cultured polyps, their diets (*Artemia salina* nauplii or the benthic copepod *Nitocra spinipes* raised on *Monochrisis lutheri* unialgal culture) and field polyps and medusae are compared. *Artemia* and *Artemia*-fed polyps have high percentages of 18:1ω9 (26.0%, 32.7%), 18:2ω6 (8.1%, 6.1%), and 18:3ω3 (32.9%, 14.7%) and low percentages of 20:5ω3 (1.3%, 1.2%) and 22:6ω3 (0%, 1.7%), respectively. 20:4ω6 (5.5% which accumulates in the polyps is low in *Artemia* (1.5%). Nitocra is low in 18:1ω9 (10.0%), 18:2ω6 (1.4%), 18:3ω3 (1.5%), 20:4ω6 (1.2%) and high in 20:5ω3 (14.5%) and 22:6ω3 (14.4%). Polyps maintained for 6 months on *Nitocra* have FA compositions more similar to those of *Artemia*-fed polyps with relatively high 18:1ω9 (26.2%) and 18:2ω6 (4.7%), but low 18:3ω3 (0.5%), 20:5ω3 (0.6%) and 22:6ω3 (2.7%). 20:4ω6 (9.6%) also accumulates in these polyps. Although the natural prey of *Chrysaora* polyps and medusae is largely unknown, phytoplankton, microzooplankton and Ctenophores are believed to be likely food organisms. FA of 1972 field polyps are similar to laboratory-reared polyps in 20:5ω3 (1.4–3.0%) but higher in 22:6ω3 (5–11%) and 20:4ω6 (12–18%). For medusae captured in 1971, the amounts of these FA are 5.2%, 13.8% and 15.6%, respectively. Polyps have approximately two-thirds shorter chain ($C_{18}$ and less) and one-third longer chain ($C_{20}$ and greater) FA, whereas medusae have one-third shorter chain and two-thirds longer chain FA.

## 61

NONMETHYLENEINTERRUPTED POLYETHYLENIC FATTY ACIDS IN MARINE INVERTEBRATES. M. PARADIS and R.G. ACKMAN, Fisheries Research Board of Canada, Halifax Lab., P.O. Box 429, Halifax, N.S., Canada.

The $C_{20}$ monoethylenic fatty acids in oysters, quahogs and some other marine invertebrates are resolved by open-tubular gas liquid chromatography (GLC) into the eicosenoic acids commonly found in marine lipids (ω11, ω9, ω7, ω5). Superimposed on these are another set of components found to be nonmethyleneinterrupted eicosadienoic acids. The docosenoic acids which appear on GLC charts on BDS are normally only ω(18 + 11), ω9 and ω7. Two obvious peaks which are identifiable as nonmethyleneinterrupted docosadienoic acids fall slightly behind the 22:1ω7, facilitating analyses in this chain length. The structures of these nonmethyleneinterrupted acids will be reviewed from the viewpoints of biochemical origin and gas liquid chromatographic behavior.

## 62

A UNIQUE ROLE FOR DOCOSAHEXAENOATE IN FISH. IAN J. TINSLEY, Dept. of Agricultural Chemistry, Oregon State University, Corvallis, Ore. 97331.

Although the linolenic acid series of PUFA have been shown to be essential for the fish, the role of individual acids of this series has not been established. This paper summarizes a number of observations made in our laboratory which suggest a unique role for docosahexaenoate 22:6 in the fish. Embryonic cells from coho salmon accumulate this acid against the concentration gradient between cells and medium, while the fingerlings will conserve this acid when starved or held at a maintenance level. Developing steelhead sac-fry tend to retain greater proportions of 22:6 than other acids, despite the fact that little if any selection is observed in fatty acid release from the yolk. Such retention could reflect a special requirement or an inability of the organism to metabolize this fatty acid. The former alternative is favored, in that this selectivity appears to be lost under certain stress conditions. In coho salmon pentachlorophenol stimulated the catabolism of all fatty acids to the same degree and exercise at high swimming velocities resulted in losses of 22:6 which were in excess of those observed for other fatty

acids. Statistical analysis has indicated that, in contrast to the other fatty acids, 22:6 is accumulated in coho salmon in relation to the size of the fish rather than the length of time they were held on a specified diet. These data suggest a special role for 22:6 in the fish possibly similar to that of arachidonate in the rat.

## 63

LIPID METABOLISM IN RELATION TO ABSORPTION, TRANSPORT, STORAGE AND MOBILIZATION OF PESTICIDES IN MAMMALS: A REVIEW. JEAN HIMMS-HAGEN, Dept. of Biochemistry, University of Ottawa, Ottawa, Ont., Canada.

Many pesticides are lipid-soluble and are stored in the mammalian body primarily in the adipose tissue triglyceride stores. The principal source of the stored pesticides is the diet, particularly lipid-containing foods of animal origin. There appears to be biological magnification of pesticides as they pass through the food chain to accumulate finally in man and other mammals. Because of their lipophilic nature pesticides may be expected to move with lipids in the normal course of their transport and metabolism. This review will consider the normal movements of lipids within the mammalian body, starting with digestion and absorption and continuing with transport in the blood, storage, mobilization and metabolism.

## 64

ROLE OF LIPIDS IN DRUG DETOXICATION. R.M. WELCH, Medicinal Biochemistry, the Wellcome Research Labs., Burrought Wellcome Co.

Abstract not available at press time.

## 65

MOBILITY OF LIPOPHILIC PESTICIDES IN AN ANIMAL. G.M. FINDLAY, Dept. of Entomology, University of Manitoba, Winnipeg, Man., Canada.

The lipids of any tissue, especially adipose tissue, are a storage reservoir for lipophilic pesticides. When located in the lipid reserves the pesticides cannot exert their lethal effects upon the central nervous system or their sublethal effects in such tissues as the liver and eggshell gland. However any condition or treatment that promotes the mobility of lipid from adipose tissues will concomitantly accelerate the movement of the pesticides into the circulation. These mobilized residues are an internal dose that can reach the sensitive tissues or be removed from the body by the excretory organs. Several researchers have attempted to accelerate pesticide excretion by starvation-induced lipid mobilization. Other workers have shown that a significant proportion of the pesticide residues mobilized from adipose tissue become relocated in other body tissues, notably the brain and muscle tissue.

## 66

MODES OF ACTION OF LIPOPHILIC PESTICIDES AT THE SUBCELLULAR LEVEL. W. CHEFURKA, Research Institute, Canada Agriculture, London, Ont., Canada.

Abstract not available at press time.

## 67

THE IMPORTANCE OF BEING LIPID SOLUBLE. D.J. ECOBICHON, Dept. of Pharmacology, Dalhousie University.

The pharmacokinetic characteristics of lipoid-soluble drugs include peculiar distribution patterns in the organism, persistence and accumulation in the body's tissues, and low rates of degradation and excretion. The agents can be highly concentrated in vivo with no overt signs of toxicity. Such symptoms may appear much later, secondary to some other physiological or disease process. Organochlorine compounds are typical of this class of agents.

## 68

INTRODUCTION TO MILK LIPIDS SYMPOSIUM. LLOYD M. SMITH, Dept. of Food Science and Technology, University of California, Davis, Calif. 95616.

Milk lipids, often referred to as "milk fat," have been of interest to man for centuries. Milk fat is a major component of bovine milk and most dairy foods, and has the highest economic value of any of the milk constituents. In 1970, over 4 billion pounds of milk fat were produced by 12 million cows in the U.S. Practically all this fat was used in food products, and provided ca. 17% of the total visible and invisible fat consumed by Americans. Although milk fat has been the subject of many investigations in the past, there is continuing interest in its complex composition and in its chemical, physical and nutritional properties. The purpose of this symposium is to review contemporary knowledge of milk lipids and to focus attention on recent developments of interest to individuals associated with the field of edible fats and oils.

## 69

ORIGIN OF THE MILK FAT GLOBULE. STUART PATTON, 105 Borland Lab., University Park, Pa. 16802.

Milk fat globules originate as fat droplets within the lactating mammary cell. These droplets are composed largely (>98%) of glycerides. Their constituent fatty acids are derived by lipolysis of very low density lipoproteins and chylomicrons of the blood and by de novo synthesis within the cell. Evidence of two principal routes for the synthesis of milk fat triglycerides has been presented: the so-called glycerol phosphate and monoglyceride pathways. Neither of these has been rigorously demonstrated in the intact lactating animal. The nature of the milk triglycerides with their unique compliment and distribution of short chain fatty acids appears to depend upon a closely regulated relation between the soluble multienzyme complex which synthesizes the fatty acids and the glyceride synthetase which is bound to the endoplasmic reticulum. The resulting triglyceride appear to self-assemble into droplets from the surface of the endoplasmic reticulum. No special ultrastructures (transport particles, vesicles, etc.) have been detected in relation to this process. Milk fat droplets at the time of secretion average several microns in diameter, there being species variations. The basic secretion mechanism involves envelopment of the droplet in plasma membrane and expulsion of it from the cell. As a consequence there are at least two pools of polar lipids (cholesterol and phospholipids) associated with secreted milk fat globules, i.e., one from the plasma membrane and one entrained earlier from the endoplasmic reticulum at the time of triglyceride synthesis and accumulation. In all, the polar lipids do not make up more than 1–2% of the total lipids in milk and a substantial fraction of them has been identified recently with plasma membrane fragments occurring in the skim milk phase. From radiotracer and ultrastructural studies this membrane material does not result simply by shedding of surface from milk fat globules. This dispersed material and the lining around milk fat globules constitute valuable sources for the study of cell membranes.

## 70

COMPOSITION OF MILK LIPIDS. ROBERT G. JENSEN, Dept. of Nutritional Sciences, University of Connecticut, Storrs, Conn. 06268.

Recent findings on the composition of milk lipids, including classes of lipids present and fatty acid composition of glycerides and phospholipids, will be presented and discussed. Emphasis will be placed on the methods employed, problems involved, validity of the results and identification of the compounds. Reference will also be made to the composition of "protected" milk lipids.

## 71

TRIGLYCERIDE STRUCTURE OF MILK LIPIDS. A. KUKSIS, Banting and Best Dept. of Medical Research, 112 College Street, Toronto 101, Ont., Canada.

Except for whale and seal, milk triglycerides of mammals differ radically from body fats. This is apparently due to difference in biosynthetic mechanisms and in composition of the available fatty acid pools. The greatest discrepancies between the depot and milk fats are seen in the ruminants, which contain 20–30 mole% of short and medium chain length fatty acids in their milk triglycerides. Although the content of these acids is much lower in man and other nonruminant mammals, they also possess characteristic positional distribution and molecular association in milk fats, when compared with the fats of other tissues, as can be revealed by chromatographic fractionation and stereospecific analysis. Although it is now possible to complete the identification of individual molecular species of triglycerides including enantiomers, this enormous task may not be necessary to assess the general structure of the milk fats and the mechanism of their biogenesis. It may be shown that the basic features can be demonstrated by determination of the structure and route(s) of biosynthesis of the 1,2-diacyl-3-butyryl-*sn*-glycerols, which can be readily isolated from ruminant milk fats. Aanalysis of the structure and specific activity of these triglycerides by combined application of argentation thin layer and gas chromatography coupled to mass spectrometry will be discussed in relation to work with molecular distillates of native and rearranged bovine milk fat triglycerides as model compounds.

## 72

CONTROL OF CHLOROPHYLL CONTENT IN SWEDISH RAPESEED AND ITS IMPORTANCE FOR THE QUALITY OF THE OIL. JOSEF DAHLEN, The Swedish Oil Extraction Co. Ltd., Karlshamn, Sweden.

The importance of the degree of maturity of rapeseed on the quality of rapeseed oil was not subjected to serious discussion and examination in Sweden until the middle of the sixties. In 1966, as a result of this discussion, seed delivered to the Swedish Extraction Association (now known as the Swedish Oil Extraction Co. Ltd.) was subjected to a continuous control and grading process. Experience gained with this process led the Swedish Oilseed Association (the growers' sales organization) to introduce a control and price regulation system, based on the chlorophyll content of the seed. Starting with the harvest of 1970, this was applied to all deliveries of seed to country elevators. These control measures, together with the grading system, have proved beneficial to oil quality, and have also led growers to show an increased interest in detecting and counteracting quality defects at an early stage.

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GLUCOSINOLATE-FREE FLOURS AND ISOLATES FROM RAPESEED AND RELATED SPECIES. H. KOZLOWSKA and F.W. SOSULSKI, Dept. of Crop Science, University of Saskatchewan, Saskatoon, Sask., Canada.

Processes for the aqueous and ethanolic extraction of glucosinolates from rapeseed meal, ground seed and the intact seed have been evaluated. All methods were relatively efficient in the detoxification of rapeseed but only the diffusion extraction of intact seed was effective in controlling the losses of oil and meal. The influence of solvent temperature, time and pH on the inactivation of the enzyme, myrosinase and the rate of glucosinolate diffusion are discussed. The yields of rapeseed flour and isolate and their protein contents were relatively low. A survey of *Brassica* species demonstrated that yellow mustard was the best source of high protein flours with low crude fiber content and isolates which had a desirable creamy appearance. The potential for breeding rapeseed with improved protein characteristics will be considered.

## 74

USE OF FATTY ACID PATTERNS TO DETERMINE FEEDING RELATIONSHIPS IN THE SEA. H. PERRY JEFFRIES, Graduate School of Oceanography, University of Rhode Island, Kingston, R.I. 02881.

This method accounts for the pattern of fatty acids in an animal's digestive tract. By relating the composition of material ingested to potential food sources, we can calculate what the dietary mixture must have been. It is a useful approach in coastal areas, where detritus is usually more abundant than phytoplankton but extremely difficult to trace in the food web. The procedure is based on total fatty acid flows rather than on specific markers. One application—to an omniverous fish—showed that the diet consists of five parts detritus to one part invertebrate tissue. A current study indicates that juvenile menhaden ingest proportionately more detritus the further they move into an estuary. In the future we intend to compare amounts of detritus eaten by a series of bivalve molluscs, each species having a progressively greater affinity for the estuarine environment. The

method of accounting for total flows, when elaborated to include the isoprenoid tracers of phytol metabolism, might then be extended to the really difficult problems of marine ecology, e.g., the feeding of deep sea animals and the nutrition of coral reefs.

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JEDDORE HARBOUR AND ITS FATTY ACIDS: RESULTS OF A SURVEY FOR HYDROCARBONS AND FATTY ACIDS IN THE WATER AND AN EXPLANATION FOR THE ODD CHAIN FATTY ACIDS FOUND IN SMELT. M. PARADIS and R.G. ACKMAN, Fisheries Research Board of Canada, Halifax Lab., P.O. Box 429, Halifax, N.S., Canada.

The hydrocarbons and fatty acids in water from Jeddore Harbour showed moderate seasonal cycles corresponding to the local eelgrass growth and contributions from marine algae. No dominant odd chain fatty acid or alcohol could be found to explain the unique occurrence of odd chain fatty acids in smelt taken in mid-winter from this locality. The source has, however, been traced to a particular stage in the life cycle of the amphipod *Pontoporeia femorata*.

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FATTY ACIDS IN NATURALLY OCCURRING ORGANISMS AND TISSUES OF RAINBOW TROUT PLANTED IN AN ALKALINE PRAIRIE LAKE IN CANADA. M. YURKOWSKI, JO-ANNE TABACHEK and HELEN BOESE. Canada Dept. of the Environment, Fisheries Research Board of Canada, Freshwater Institute, Winnipeg, Man., Canada R3T 2N6.

In the prairie provinces of Canada, there are many small highly productive lakes, which appear to be suitable for fish farming. From Nora Lake near Erickson, Manitoba, a sample was taken in late August of the following organisms: (1) planktonic algae (primarily *Aphanizomenon*); (2) planktonic crustaceans (contained 75% copepods, *Diaptomus sicilis*, and cladocerans, 25% *Daphnia schoedleri* and 5% *Diaphanosoma leuchterbergianum*; (3) amphipods (*Gammarus lacustris*); (4) adult salamanders (*Ambystoma tigrinum diaboli*, Duncan); and (5) liver, carcass and kidney from rainbow trout (*Salmo gairdneri*), which had been planted as fingerlings 3 months previously. The concentration and fatty acid composition of the total neutral lipids and total phospholipids from the organisms were compared. The trout liver and kidney and salamanders contained 2.6–3.7%; trout liver 7.9% and the other organisms 0.3–1.3%, neutral lipids. The liver and kidney from trout contained 1.6 and 3.2% phospholipids, respectively. The other samples contained 0.3–1.0%. The fatty acid distribution of these organisms and trout tissues were similar in most cases. However in the neutral lipids there were higher levels of 18:1 in trout tissues, 16:1 in amphipods, and lower levels of 18:3 in trout tissues and 22:6 in amphipods, than those in the other organisms. The level of 18:1 in the amphipods and salamanders was intermediate to that of the trout tissues and the other organisms. In the phospholipids there were higher concentrations of 14:0 and 18:3 in algae, 18:2 in algae and amphipods, 18:1 in salamanders and amphipods, 16:1 in crustaceans and 22:5 (n-3) and 22:6 in trout tissues than those in the other organisms. However there were lower concentrations of 20:5 in the liver and algae, 20:4(*n*-6) in the algae, 18:3 in the liver, 18:2 in the trout tissues and 18:0 in algae and amphipods. The trout tissues contained considerably more 22:6 than 20:5 and the reverse was observed in the amphipods and salamanders.

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FATTY ACIDS IN AN INTERTIDAL POLYCHAETE: THEIR RELATION TO THE MARINE FOOD CHAIN AND TO THE PHYSIOLOGY OF THE WORM. DOROTHY M.-E. POCOCK and JOAN RATTENBURY MARSDEN, University Medical Clinic, Montreal General Hospital, 1650 Cedar Ave., Montreal 109, Que., Canada, and James G. Hamilton.

Fatty acids of triglycerides, alkenyl and alkyl glyceryl ether diesters, total neutral lipid fractions and phospholipids in *Nereis virens*, and fatty acids in mussels from the same beach, were analyzed by gas liquid chromatography (GLC). Palmitic, oleic, eicosaenoic, eicosapentaenoic and docosadienoic acids predominated in the depot lipids of *N. virens*. The proportion of monoenes decreased in the phospholipids concurrent with an increase in eicosapentaenoic acid. Docosahexaenoic acid was noticeably absent in all the different lipid fractions; however docosatetraenoic and docosapentaenoic acids did occur. Therefore the fatty acid spectrum of *N. virens* was typically marine except for the 22 carbon fatty acids: 22:6 was absent; the amount of 22:2 exceeded the amount of 22:1, rose in starved animals and exceeded the amount of 20:5 in mature male coelomic fluid; 22:4 and 22:5 seemed to be significant in the maturation of males and females, respectively. The mussels contained docosahexaenoic and docosadienoic acids. The absence of docosahexaenoic acid in *N. virens* may reflect phylogeny because the tropical marine worm also analyzed did not contain docosahexaenoic acid either. On the other hand, the *docosadienoic* acid in both *N. virens* and the mussels may reflect dietary similarities. Although the mussel is a filter feeder and *N. virens* is a detritus feeder, microorganisms available to the mussel will also contribute organic matter to the mud that *N. virens* ingests. Dimethyl acetals were major components in the fatty acid methyl ester fractions prepared from isolated alkenyl glyceryl ether diesters. A fatty acid that behaved on GLC like a hexadecapolyene increased dramatically in total neutral lipid fractions from all worm tissues during starvation but increased in phospholipids only from coelomic fluid during starvation. This compound was not found in isolated triglycerides or glyceryl ether diesters, and it may be indigenous to microorganisms in the coelomic fluid of the worm rather than to *N. virens*.

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7-METHYL-7-HEXADECENOIC ACID IN THE OCEAN SUNFISH *Mola mola*. R.G. ACKMAN, S.N. HOOPER and M. PARADIS, Fisheries Research Board of Canada, Halifax Lab., P.O. Box 429, Halifax, N.S., Canada.

The origin of the 7-methylhexadecanoic acid found in many marine oils is probably associated with 7-methyl-7-hexadecenoic acid. This acid has been isolated from the liver oil of the ocean sunfish where it is found in an exceptionally high proportion. Comparisons will be made with monomethyl-branched acids from other animal sources.

## 79

COMPARATIVE FATTY ACIDS OF PACIFIC TELEOST FISH. JAMES B. SADDLER, Drawer LW, State College, Miss. 39762.

Teleost fish residing in the coastal streams of Washington, northwest Pacific Ocean and in the Berring sea off the coast of Alaska exhibit many different environmental conditions and feeding habits. They do share, however, some similarities and differences in total liver lipids and fatty acid percentages. Five species of salmonid fishes in the northwest Pacific contain an average of 5% lipid in the liver, whereas the shad, lingcod, sablefish and rockfish vary from 6–20% lipid in their liver tissue. In contrast, their muscle tissue lipids are the opposite, salmonids an average of 20% and the lingcod 1–2% lipid. There is no major difference in average total lipids of the salmonids taken off the coast of Washington to those taken from the Bering Sea. To contrast, the percentage of Oleic acid found in the liver of the lingcod was over 50% of the total, while the percentage found in salmonids was 15–18% of the total. Docosahexaenoic acid in salmonids averaged 25% of the total while the percentage in the shad was 5% and in the lingcod 10% of the total. Salmonids in freshwater streams whether young migrating to sea or adults returning to the streams to spawn also display percentage changes in docosahexaenoic acid. In the streams, salmonids both young and adult average 15%, but in salt water this percentage of the total increases to 25%. Salmonids fed pellet diets prior to release also show decreased percentages of 22:6, but in an average of 10 days after release have 25% of (22:6). Type and availability of food may account for some of the change; however the liver tissue also reflects other nutritional involvements that may reflect lipid retention or depletion. Both freshwater and saltwater fish may vary lipid content in different sections of the organ.

## 80

MONOETHYLENIC ISOMERS IN CARDIAC LIPIDS OF RATS FED PARTIALLY HYDROGENATED HERRING OIL. H.B.S. CONACHER, B.D. PAGE and J.L. BEARE-ROGERS, Research Lab., Health Protection Branch, Tunney's Pasture, Ottawa, Ont. K1A 0L2, Canada.

In previous experiments it was demonstrated that the feeding of partially hydrogenated oils containing $C_{22}$ acids to young rats resulted in increased deposition of cardiac fatty acids. Little however was known of the geometrical and positional monoenoic isomers in the deposited fatty acids in relation to those present in the oil fed. To gain this information, compositional studies have been carried out on a partially hydrogenated herring oil and on the cardiac lipids of young rats fed this oil for 1 week (peak lipid deposition) and after prolonged feeding (16 weeks). Deposited isomers were compared to those present in the original oil and evidence of $\beta$-oxidation observed.

## 81

WAX ESTERS IN BARRACUDINA. R.G. ACKMAN, C.A. EATON and S.N. HOOPER, Fisheries Research Board of Canada, Halifax Lab., P.O. Box 429, Halifax, N.S., Canada.

Barracudina are seasonally available in large numbers off the east coast of Canada. In 1971, total lipid was of the order of 18%, of which 85% was wax ester and 10% triglyceride. The wax ester fatty acids had a "normal" marine oil composition, but the fatty alcohols included octadecenols, eicosenols and docosenols in addition to the major (42%) hexadecanol component. The triglycerides were of exceptionally low iodine value (ca. 48). This oil will be discussed in terms of a replacement for sperm whale oil. Additional data from the 1972 season will be presented.

## 82

PHOSPHOLIPID ACTIVITY IN THE BRAIN OF A POLYCHAETE WORM, *Nereis virens*. JOAN MARSDEN, P.O. Box 25208, Nairobi, Kenya, East Africa.

Seven phospholipids have been found in the supraoesophageal ganglion, or brain, of the polychaete worm, *Nereis virens*. Of these phosphatidyl choline is the most abundant, although phosphatidyl ethanolamine occurs in almost equal quantity. Both vinyl-ether and diacyl forms of phosphatidyl ethanolamine are present in approximately equal amounts. Phosphatidyl inositol ranks third in abundance. The incorporation of 32 P. orthophosphate by these brain phospholipids varies with the age and sexual maturity of the worm. Phosphatidyl inositol, for instance, at 6 hr after injection of 32 P. orthophosphate, is labeled emphatically in young worms, lightly in older individuals and is not labeled at all in mature animals. The same sort of decline in activity with increasing size and age has been shown for each of six brain phospholipids and is believed to reflect a decline in rate of growth. However there is in addition a more profound change in the activity of the phosphatidyl inositol moiety as the animal ages. The exceptionally steep decline in rate of phosphatidyl inositol synthesis with age, as compared with changes in other brain phospholipids, suggests a progressive reduction of some particular biochemical activity in the brain. In nereid worms the brain is very much an endocrine as well as a nervous organ. Its best documented secretion, a neurosecretion, is a juvenile hormone which is produced in quantity only in young, immature animals. This secretory activity declines as sexual development begins and ceases at sexual maturity. Also a very high level of phosphatidyl moistol synthesis is known to be symptomatic of secretion in a number of mammalian glandular tissues. It is suggested here that the demonstration of high levels of phosphatidyl inositol activity which declines to zero at sexual maturity is a direct reflection of neurosecretory activity in the brain of *Nereis virens*.

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PROGRESS IN THE STRUCTURAL ELUCIDATION OF FATTY ACIDS AND TRIGLYCERIDES THROUGH THE USE OF CHEMICAL SHIFT REAGENTS AND NMR SPECTROSCOPY. PHILIP E. PFEFFER, E. Market. Nutr. Res. Div., ARS, USDA, 600 East Mermaid Lane, Philadelphia, Pa. 19118.

Innumerable publications concerned with the structural elucidation of molecules via lanthanide shift reagents and NMR spectroscopy have appeared since Hinckley's initial discovery of the potent effects of the trivalent europium complex. Europium, as well as other members of the lanthanide series in the form of β-di-ketone complexes (shift reagents), produce an apparent anisotropic effect on the resonances of compounds containing *such interacting polar groups as* OH, $NH_2$ or O ‖ C–OR. Compounds which normally exhibit featureless spectra, in the presence of shift reagents, display discrete resolvable resonances which are amenable to a simple first order analysis. Initial spectral studies of saturated fatty acid esters and alcohols were limited in application, since the shift reagent only allowed for resolution of the proton resonances along the chain up to the sixth carbon. However more recent investigation has shown that the presence of a double bond at the 6,7 position of the fatty acid ester chain provides additional structural information by extending the effect of the shift reagent to the eighth carbon. Furthermore, if a second polar substituent such as a hydroxyl or epoxy group is located near position twelve in the chain, a complete first order spectrum of a $C_{18}$ hydroxy or epoxy ester may be revealed. Analysis of mixtures of fatty acid esters has also become possible through the use of shift reagents since each complex formed between the ester and shift reagent has its own characteristic formation constant $K_f$. By taking advantage of the differences of the various $K_f$'s, isomeric esters such as oleate and elaidate as well as mixtures of aliphatic saturated materials may be analyzed directly. Triglycerides are also amenable to the analysis of their structures by the complexation technique. Investigation of partially unsaturated triglycerides has indicated that the position of the unsaturated chain in the glyceride molecules allows for discrimination of isomeric symmetrical and unsymmetrical triglycerides. Detailed deuterium labeling experiments have been carried out to substantiate these structural and proton resonance assignments. Analysis of mixed chain length and branched chain triglycerides has also been explored.

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COMPUTER IDENTIFICATION OF FATS AND OILS FROM THEIR FATTY ACID COMPOSITION. S.F. Herb and Paul J. Gormisky, E. Reg. Res. Lab., E. Market. Nutr. Res. Div., ARS, USDA, 600 East Mermaid Lane, Philadelphia, Pa. 19118.

Widely divergent fatty acid compositions have been published for most fats and oils. Identification has been proposed for 11 of the more common ones from their fatty acid composition as analyzed by gas liquid chromatography. Their fatty acid specifications are under consideration for adoption by the Codex Committee on Fats and Oils of the Joint Food and Agricultural Organization of the United Nations and the World Health Organizations. The specifications have been incorporated in a computer program and compared with a large number of fatty acid analyses of known oils. The results show that the correct oil was identified nearly 100% of the time. By having several specifications with narrower fatty acid ranges for each oil, it may be possible to classify an oil as originating from seeds grown in a particular country and to detect hydrogenated oils.

## 85

MICROPARTICULATE BED CHROMATOGRAPHY ACCELERATED BY CENTRIFUGAL FORCE AND PRESSURE. Edgar Ribi, Rocky Mountain Lab., Hamilton, Mont. 59840.

A chromatographic technique for separating lipids which utilizes centrifugal force for packing columns of microparticulate gel will be described. The use of such ultrafine silica composed of 150 A wide leaflets provides improved resolution. For analytical work, 3 mm diameter columns are employed, and the migration of samples is also accelerated by the application of centrifugal force. The bands formed by the sample components are detected in the microgram range by the methods used in thin layer chromatography. Resolution was not impaired when the column diameter was increased and, consequently, in preparative procedures, columns 8 or 18 mm in diameter are packed by centrifugation, and then pressure is applied to accelerate the chromatographic process. Two columns may be pressurized simultaneously, and 10 mg of sample may be applied to each column. The eluate is monitored with UV light and collected in a conventional fraction collector. If monitoring is not possible, aliquots removed from individual fractions collected are rechromatographed with the analytical apparatus. The method is distinctive in its simplicity, high resolving power, reproducibility and rapidity.

## 86

IDENTIFICATION OF LIPIDS FROM THE VAPOR PHASE THERMAL FRAGMENTATION PATTERNS OF GAS LIQUID CHROMATOGRAPHY EFFLUENTS. Eugene J. Levy, Chemical Data Systems, Oxford, Pa.

Abstract not available at press time.

## 87

EXCHANGE OF LIPOPROTEIN CHOLESTEROL WITH INTACT AORTIC TISSUE AND ISOLATED ERYTHROCYTE MEMBRANES. Frank P. Bell, Dept. of Pathology, McMaster University, P.O. Box 590, Hamilton, Ont., Canada.

The exchange of unesterified cholesterol between serum lipoproteins, aortas and erythrocyte membranes was examined under a variety of conditions, in vitro. The availability of unesterified cholesterol of erythrocyte membranes and lipoproteins for exchange was unaffected by formalin fixation. In addition, stimulation of cholesterol exchange between membranes and lipoproteins in the presence of dimethyl sulfoxide was independent of fixation. By contrast, however, fixation of aorta tissue reduced cholesterol exchange from lipoproteins by 50% and reduced dimethyl sulfoxide stimulation of cholesterol exchange 42%. The difference in cholesterol exchange behavior of isolated membranes and whole tissue in response to formalin fixation and dimethyl sulfoxide are discussed on the basis of current models for the structure of membranes and lipoproteins. In addition, a mechanism is proposed for the intracellular distribution of cholesterol exchanged to the plasma membrane of cells.

## 88

THE EFFECTS OF DIETARY CHOLESTEROL ON THE SYNTHESIS OR RELEASE OF RAT SERUM HIGH DENSITY LIPOPROTEINS. Jerome V. Franka and Raymond Reiser, Dept. of Biochemistry and Biophysics, Texas A&M University, College Station, Tex. 77843.

While there have been conflicting reports in past literature concerning the effects of dietary cholesterol on the levels of rat serum high density lipoproteins (HDL), more recent evidence indicates a significant suppression or reduction in HDL levels. Data from our laboratory indicate not only a suppression of HDL levels by dietary cholesterol but also a depression in the rate of synthesis or release. When large doses (0.1 mc) leucine-4,5 $H^3$ are injected into rats, and serum is obtained at graded intervals from 30–150 min following injection, the specific activity of apo HDL from cholesterol-free fed rats is significantly higher when compared to rats maintained on high cholesterol diets. Data consistently reflect the most pronounced differences at 30 min following injection. Preliminary data indicate that the specific activity of apo HDL from cholesterol fed rats is not as high as that from cholesterol-free fed rats until 90–120 min following injection. Although the specific activities of very low density lipoproteins and low density lipoproteins (LDL) are much higher, their differences in response to diet are insignificant when compared to differences in HDL response. A number of suggestions stem from these studies: (1) data on dietary or other effects on lipoprotein synthesis or release are not meaningful unless initial rates are compared; (2) the repression of HDL synthesis by dietary cholesterol is accompanied by a more rapid rate of LDL synthesis; and (3) the synthesis or release of cholesterol may be directly related to the synthesis of apo HDL.

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QUANTITATION OF HUMAN PLASMA LOW DENSITY LIPOPROTEINS BY SINGLE RADIAL IMMUNODIFFUSION OF WHOLE PLASMA. Richard J. King and Joe C. Christian, Dept. of Medical Genetics, 129 Riley Research Wing, Indiana University Medical School, 1100 West Michigan St., Indianapolis, Ind. 46202.

Elevated plasma low density lipoproteins (LDL) have been implicated as a risk factor in atherosclerosis. LDL may be quantitated by ultracentrifugation, precipitation, or immunological methods. Single radial immunodiffusion (SRI) was evaluated to determine if it gave more information about the quantity of LDL than is obtained by lipid analysis of whole plasma. Fasting blood was drawn from 74 male Caucasian twins, aged 45–55, and SRI performed in duplicate using commercial agar plates (Partigen Beta-lipoprotein Immunodiffusion Plates, Behring Diagnostics, Inc., Woodbury, N.Y. 11797), with 0.002 ml of plasma being placed in the precut wells and allowed to diffuse. After 8 days the square of the diameter of the precipitin ring was taken as a measure of LDL. In addition, plasma cholesterol (C), lipid phosphorus (LP) and triglycerides, plus LDL and high density lipoprotein (HDL) cholesterol, LP and protein, were measured from ultracentrifuged plasma. SRI had significant positive correlations ($P < 0.01$) with all of the total plasma and LDL variables measured. The correlations of the LDL variables were consistently higher than those of the total plasma variables. The multiple correlation coefficient (R) of SRI with these same variables was 0.79, with only LDL-PL and HDL protein contributing significantly. This R was only 0.03 greater than the simple correlation between SRI and LDL-PL, and this increase reflected a negative relationship between HDL protein and SRI. In addition to the original variables, a plasma C/PL ratio was constructed, because this ratio has been reported to be highly correlated with LDL. The C/PL ratio was significantly correlated with LDL lipids and protein ($P < 0.01$). The correlations of SRI and LDL-PL (0.76) and protein (0.64) were higher than the correlation of the C/PL ratio with LDL-PL (0.38) and protein (0.36). Correlation of SRI and LDL-C (0.67) and C/PL with LDL-C (0.67) were identical, indicating that these parameters are equally efficient in estimating LDL-C. SRI of whole plasma apparently gives a better measure of total LDL than may be obtained from plasma lipid analysis; however, the whole plasma C/PL ratio correlated equally well with LDL-C.

## 90

DYNAMICS OF CHOLESTEROL AND CHOLESTERYL ESTERS IN THE LIVER PERFUSION SYSTEM. Sandra J. Petersburg and R.D. Ellefson, Dept. of Laboratory Medicine, Mayo Clinic and Mayo Graduate School of Medicine, Rochester, Minn. 55901.

Rat serum very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL), containing $^{14}C$-cholesterol and $^{14}C$-cholesteryl esters, were prepared in vivo from $^{14}C$-mevalonic acid. The lipoproteins of each class were infused into circulating, defibrinated rat blood in the perfusion system both in the presence and in the absence of the liver. The transfers of cholesterol and cholesteryl esters from the lipoproteins of each class to the lipoproteins of other classes, to the red blood cells (RBC) and to the liver were not random but followed a definite pattern. Of all the possible transfers of cholesterol, the following were observed to occur during the 8 hr experimental period. The cholesterol of VLDL and LDL transferred directly to the RBC both in the presence and in the absence of the liver. The liver cleared cholesterol directly from VLDL and from the RBC and secreted cholesterol which appeared in the RBC. HDL cholesterol transferred to LDL. The following direct transfers of cholesterol occurred only slightly, if at all: VLDL to LDL, VLDL to HDL, LDL to VLDL, LDL to liver, HDL to VLDL, HDL to RBC, HDL to liver. The only direct transfer of cholesteryl esters in the circulating blood was from HDL to LDL. The liver cleared cholesteryl esters extensively from the lipoproteins of all three classes. Most of the cholesteryl esters cleared by the liver were hydrolyzed soon after clearance. Cholesteryl esters cleared by the liver from HDL were the apparent sources of the cholesterol secreted by the liver in newly synthesized LDL.

## 91

INVESTIGATION OF HIGH-DENSITY SERUM LIPOPROTEINS BY FLUORESCENCE AND FLUORESCENT

PROBES. NORMAN K. FREEMAN and ALEX V. NICHOLS, Donner Lab., University of California, Berkeley, Calif. 94720.

Some observations have been made of the intrinsic fluorescence of high-density serum lipoproteins and of the apoproteins obtained from them by delipidization. The effects of temperature, dehydration and denaturing agents have been studied. Quenching experiments with potassium iodide have provided an estimate of the number of surface-exposed tryptophans per molecule. Binding of fluorescent probe molecules (1-anilino-8-naphthalene sulfonate and 2-toluidino-6-naphthalene sulfonate) to both lipoproteins and apoprotein have also been investigated. Measurements of the depolarization of the fluorescence of such probe molecules indicate that they have considerable freedom of motion within the lipid regions of the macromolecules.

## 92

THERMOGRAVIMETRIC ANALYSIS OF SERUM LIPOPROTEIN FRACTIONS. FRANK T. LINDGREN and GARY R. STEVENS, Donner Lab., University of California, Berkeley, Calif. 94720.

Using a Cahn RG electrobalance, chylomicron-containing fractions and very low density lipoprotein fractions have been analyzed by thermogravimetric analysis (TGA). Some modifications in the usual procedure are needed to provide reproducibility and stability of measurement. The initial solution mass (1–3 drops) is weighed with a semimicrobalance. The dried residue is measured with a microbalance after $H_2O$ removal and after calcination at 500 C. In addition to measurement of lipoprotein concentrations with a minimum of material, the results allow accurate determination of the salt background. Combined with precision refractometry, these results potentially provide an accurate and simplified measurement of lipoprotein specific refractive increment.

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A RAPID SCREENING TEST FOR ELEVATED SERUM TRIGLYCERIDES OR CHOLESTEROL, OR BOTH. JOE C. CHRISTIAN, Dept. of Medical Genetics, 129 Riley Research Wing, Indiana University Medical School, 1100 West Michigan Street, Indianapolis, Ind. 46202.

Elevated serum cholesterol and triglycerides have been shown to be risk factors in the development of atherosclerotic heart disease, but their large scale evaluation has been hampered by the lack of reliable, rapid and inexpensive screening tests. Chemical quantitation of cholesterol and triglycerides is expensive and difficult to standardize between laboratories; lipoprotein electrophoresis is difficult to quantitate and turbidimetric methods require equipment not generally available. Ames Laboratory (Elkhart, Ind.) has developed a lipid screening test (LST) based upon a reagent designed to agglutinate low density (LDL) and very low density lipoproteins into a precipitate that can be centrifuged and then quantitated volumetrically. This paper reports the results of evaluating this screening test, done in duplicate, on fasting serum from 76 Caucasian twins aged 45–55, along with lipid analyses including cholesterol (C), phospholipids (PL) and triglycerides (T) in whole plasma as well as C, PL and protein in high density lipoprotein (HDL) and LDL fractions obtained by ultracentrifugation. The LST was done by mixing 0.1 ml aliquots of the lipid screening reagent and serum, drawing a 60 mm column of the mixture into a marked capillary tube, sealing one end of the tube and then spinning the tube 10 min in a microhematocrit centrifuge. The precipitate height was read to the nearest 0.02 mm under a microscope fitted with a micrometer. The LST precipitate ranged from 0.76–5.96 mm (mean = 1.48, standard deviation = 0.82) with a laboratory error standard deviation of 0.05 between duplicate determinations. The LST values were correlated with the lipids measured and significant correlations ($P < 0.01$) were found with plasma-C, PL and T as well as LDL-C, PL and protein (the largest was plasma-T, 0.83); there were no significant correlations ($P > 0.05$) with any HDL variable measured. A multiple correlation of LST with the other variables revealed that only plasma-C and T contributed significantly giving a multiple correlation coefficient of 0.90. This LST should be useful in screening for individuals with elevated cholesterol or triglycerides, or both. Measurement of plasma cholesterol in individuals found to have an elevated LST allows calculation of plasma triglycerides. A nomogram has been developed to simplify this calculation.

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CRYSTALLIZATION AND COMMERCIAL FRACTIONATION OF MILK FAT: A REVIEW. JOHN W. SHERBON, Dept. of Food Science, Stocking Hall, Cornell University, Ithaca, N.Y. 14850.

This selective literature review covers recent progress in understanding milk fat crystallization and fractionation. The extent of fat crystallization in butter may be increased by low temperature incubation or rapid cooling of cream prior to churning. It may be decreased by initiation crystallization at a low temperature, then incubating above final churning temperature. Pure triglycerides exhibit monotropic polymorphism, but it is more difficult to demonstrate the same phenomenon in milk fat because of solid solution formation. The nature of solid phases in binary and tertiary mixtures of triglycerides is used to illustrate solid solution formation in milk fat. Modification of crystallization by addition on surfactants to the milk fat is discussed. The major high melting fraction of milk fat is the HMGF first reported by Jenness and Palmer. This material can be fractionated further to give several highly saturated fats having melting characteristics of pure phases. The composition and properties of fractions obtained by progressive fractional crystallization from either the melt or solutions are affected by conditions such as size of cooling step and concentration and composition of fat in the supernatant. Commercial fat fractionation processes are described and typical results obtained by the Alfa-Laval method are presented. Applications for fractionated milk fat are briefly mentioned.

## 95

SOME FACTORS AFFECTING HYDROGENATION OF MILK FAT. LLOYD M. SMITH and ANDRES VASCONCELLOS, University of California, Davis, Calif.

Hydrogenation of milk fat with palladium and nickel catalysts was studied at various temperatures, pressures and concentrations of catalyst. Samples were removed from the laboratory hydrogenator at intervals during the reaction, and changes in refractive index, iodine value, Wiley melting point, and percentage of trans isomers were determined. Palladium was several times more active as a catalyst than was nickel. Milk fat with an iodine value of 35 and melting point of 34 C was hydrogenated with 0.05% palladium (active catalyst) to an iodine value of <5 and a melting point >49 C, in 30 min at 121 C and 53 psi of hydrogen. Kinetic data for each catalyst yielded two slopes, indicating two reaction rates were operative.

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METHODS FOR THE ISOLATION AND CHARACTERIZATION OF TRACE COMPONENTS FROM MILKFAT. D.P. SCHWARTZ, USDA, E. Market. Nutr. Res. Div., South Building Room 1628, Washington, D.C. 20250.

New methods have been developed for isolating the trace amounts of keto fatty acids, hydroxy fatty acids, glyceryl-1-alkyl ethers, fatty aldehydes, sterols and other alcohols present in milkfat. The classes are isolated as colored derivatives. They are separated into saturated and unsaturated classes by argentation chromatography and further resolved by adsorption and liquid-liquid partition chromatography. Identification of compounds is made by thin layer chromatography or by gas liquid chromatography-mass spectrometry, or both, after regenerating the parent compound. Liquid-liquid micro reaction columns have been used in some phases of the overall scheme. These are columns in which one of the reactants is dissolved in water and impregnated onto an inert support. The other reactant dissolved in a water-immiscible solvent is passed over the column. Reaction takes place on the surface of the support, and the product(s) of the reaction emerge continuously. The following classical reactions have been adapted to microcolumn procedures: periodic acid and chromic acid oxidations, removal of aldehydes with sodium bisulphite, location of double bond position, selective extraction of individual fatty acids and the removal of 3$\beta$-sterols by digitonin.

## 97

NUTRITIONAL VALUE OF FATS. FRED H. MATTSON, The Procter & Gamble Co., Miami Valley Lab., P.O. Box 39175, Cincinnati, Ohio 45239.

Extensive studies, particularly those by Harry Deuel, established that the usual dietary fats are equivalent in overall nutritional value. In recent years interest in dietary fats has centered on their role in more specific biological systems. Such studies have been made possible, in part, by new synthetic and analytical techniques. Thus the absorbability of a fat has been shown to be determined not only by its content of certain fatty acids but also by the specific structure of the triglycerides making up the fat. Specific fatty acids have been shown to be related to the accumulation of triglycerides in the heart of experimental animals and to the alteration of the level of cholesterol in the blood of experimental animals and humans. The optimal dietary levels of cholesterol and tocopherol have been related to other environmental factors. Observation of these types have led to recommendations for changes in the level and in the composition of the fats consumed in Canada and the US. To become operative, recommendations alone are not sufficient; such changes (1) must be adaptable to established eating patterns; (2) if they result in major economic dislocations, these must be recognized and deemed to be acceptable; (3) if a food item is to be consumed in increasing quantities, it must be in adequate, or potentially adequate, supply at a reasonable cost; and (4) must not result in dietary inbalances of other, essential nutrients.

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THE ROLE OF FOOD LIPIDS IN HUMAN NUTRITION. M.A. AMER and G.J. BRISSON, Nutrition Research Center, Laval University, Queb., Canada.

Approximately 40% of the calories in the normal Canadian diet are derived from fats, which is about equal to the calories derived from carbohydrates. As fat can readily be synthesized in abundant amounts from carbohydrate and protein, it was earlier considered to be a dispensable component of the diet. According to modern opinion, apart from the vital role that fat plays in metabolism, it is important in the diet as carriers for certain necessary nutrients, especially fat soluble vitamins. Certain unsaturated fatty acids are essential for good nutrition and protection against development of many undesirable conditions. The understanding of the role of essential fatty acids in nutrition and in metabolic process is far from complete. Both the amount and type of fat in the diet have an important effect on serum lipid levels which in turn may have an influence on the development of atheroma, atherosclerosis, hyperlipemia and obesity. Nutritional advantages have been claimed for various fats, especially for butter against margarine or for animal rather than vegetable fats. The question of the optimal level of fat in the diet is complex. It has been suggested that fat should provide 20–25% of the total calories and that essential fatty acid should account for at least 1% of the total calories. The clearer definition of these optimum levels is a matter of great importance.

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CONTROL OF BLOOD LIPID LEVELS. PAUL-J. LUPIEN, Lipid Disease Research Center, Center Hospital, Laval University, Queb., Canada.

In the past decade interest in lipids, their determination and their alterations in many diseases has increased to such an extent that a new medical specialty has evolved. Perhaps one of the most interesting aspects of such studies has been those related to our understanding of the regulatory mechanisms which mediate lipid homeostasis in the organism. Such studies have demonstrated that dietary management as well as various pharmacological agents could be used successfully to lower blood lipid levels in certain types of hyperlipidemia. It is the purpose of this presentation to review briefly some of the effects of known hypolipidemic agents as well as the possible application of a newer compound, 3—OH—3—$CH_3$ glutaric acid, in maintaining normal lipid levels.

## 100

EFFECT OF DIET ON THE COMPOSITION OF BODY FAT. GERMAIN J. BRISSON, Nutrition Research Center, Laval University, Queb., Canada.

About 80% of the fat consumed in the average North American diet comes from three major food groups, which are dairy products, meat, fat and oils. Fat of animal origin constitutes ca. 66% of total fat consumed and therefore is preponderant. On a weight basis, fatty acids constitute ca. 92–95% of the natural triglycerides, and as a consequence the nature of these fatty acids is responsible for the physical, chemical and physiological properties of food fat. The fatty acid composition of animal fat has received much attention during recent years. Animal scientists have been quite concerned with the bad publicity given to animal products with regard to cardiovascular diseases. Although no direct evidence could be put forth to support or justify such adverse publicity, studies were initiated to investigate the factors responsible for the chemical constitution of animal fats. Among these factors, age of animal, sex, species, hormone treatment, anatomical location of fat depot, season of the year and diet consumed have been studied. Of these factors, however, dietary treatments have received the most attention and therefore will be discussed thoroughly. In general the fatty acid composition of fat depot in monogastric animals is under the influence of the type of lipids consumed and, within certain limits, poultry meat and pork could be produced with specified fatty acid patterns, if this were found desirable. The situation is somewhat different with adult polygastric animals, which contribute a great deal to the production of food fat in the form of milk, butter, cheese and red meat. Attempts made to modify the fatty acid composition of these products through dietary means will be presented.

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MICROBIAL LIPIDS. J. GOULET and A.C. BLACKWOOD, Dept. of Microbiology, Macdonald College of McGill, Macdonald Coll. P.O., Queb., Canada.

That some microorganisms can store fat has been known for some time; however extensive studies dealing with the accumulation of lipid by microorganisms started only in the late forties when Enebo et al. (1948) described a particular yeast which could accumulate at least 50–60% of its dry weight as lipid. Other investigations have shown that it is also possible to convert, with relatively good yields, such low cost substrates as *n*-alkanes into specific high price fatty acids; several problems like the transformation of steroids, the desaturation of saturated fatty acids, and the biosynthesis of liposoluble vitamins are other examples illustrating the usefulness of microorganisms in the field of lipid chemistry. Since pollution problems are becoming more and more crucial and are intimately related to economics, more and more interest is shown in treating wastes in such a way that valuable byproducts can be recovered. From such a consideration, we have started a project on the biosynthesis of lipids by a pink yeast, *Rhodotorula glutinis*, on industrial effluents using both batch and continuous cultures. Different effluents, mainly from food industries, have been tested along with the influence of such factors as pH, temperature and aeration on the composition of the lipid fraction of the organisms. The fatty acid composition of the microbial fats was found to be highly unsaturated, with oleic acid as the main constituent. Linoleic, linolenic and palmitoleic acids were also detected. In the saturated fraction, palmitic and stearic acids were by far the most important constituents. The composition of the different fractions was also shown to vary with the stage of growth of the organism.

## 102

EVALUATION OF A GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF FOOD TOCOPHEROLS. HAL T. SLOVER and JOANNA LEHMANN, Human Nutr. Res. Div., ARS, USDA, Beltsville, Md. 20705.

A gas chromatographic method for the determination of tocopherols (Vitamin E) was previously reported. It has now been tested on a variety of foods by two independent laboratories. Each laboratory analyzed in duplicate aliquots of 38 different foods including grains (corn, barley, oats, rye, wheat and rice), nuts (almonds, walnuts, peanuts, pecans and cashews), vegetables (asparagus, broccoli, cabbage, carrots, cauliflower, beans and spinach), whole soybeans and sunflower seeds for alpha, beta, gamma and delta tocopherol and alpha, beta, gamma, and delta tocotrienol. An evaluation of the results, critique of the method and compositional data will be presented. The proximate composition of each sample was also determined.

## 103

INTERPRETATION OF THE ELECTRON SPIN RESONANCE SPECTRA OF LIPID SPIN PROBES IN HYDRATED LIPID MULTIBILAYERS. IAN C.P. SMITH, K.W. BUTLER, R.D. LAPPER, S. SCHREIER-MUCCILLO and D. MARSH, Div. of Biological Sciences, National Res. Council of Canada, Ottawa, Ont., Canada K1A OR6.

The electron spin resonance (ESR) spectra of spin probes are very sensitive to the orientation and mobility of the probes. Probes which are very similar in structure to naturally occurring lipids can thus be used to monitor lipid regions of real and model membranes. The types of motion and degree of organization observed depend on the nature of the spin probes and the composition of the lipid system. We have suggested models for the states of the spin probes in a series of lipid systems, and verified them by simulating the ESR spectra on a digital computer. Steroid spin probes and fatty acid spin probes (with nitroxide spin labels at various positions along the fatty acid chain) in lecithin films undergo a rapid random walk within a cone of restricted dimension, the axis of which is perpendicular to the plane of the bilayers. On the average the long axes of the probes are perdendicular to the bilayer plane. Addition of cholesterol to egg lecithin films tends to decrease the amplitude of the cone and the rate of the motion to a minimum at 55 mole% cholesterol. The probability of trans-gauche isomerizations along the hydrocarbon chains is also seen to decrease as the cholesterol concentration increases. In dipalmitoyl lecithin films the amplitude and rate of the motion increase with increasing cholesterol content. In multibilayers of lipids from bovine brain the only motion manifest in the ESR spectra is rapid rotation about the probe long axis. There is a wide distribution of orientations of these long axes which narrows with increasing cholesterol content. The addition of cholesterol produces similar effects on both oriented multibilayers and liposomes. The fatty acid probes show a higher degree of order in the latter, revealing differences in lipid organization in the two systems. Comparison of the conclusions reached from the spin probe data with those available from NMR studies on similar systems demonstrates that the spin probes are faithful indicators of the organization and mobility of the membrane lipids themselevs.

## 104

SPIN LABEL STUDIES OF MEMBRANE STRUCTURE. J.C. HSIA, Dept. of Pharmacology, Faculty of Medicine, University of Toronto, Toronto 181, Ont., Canada.

Biological membranes assembled with various specific proteins and lipids are capable of regulating their intracellular environment and responding to external chemical and physical stimulations. Our understanding of the structure and function relationship of biological membranes depends upon detailed knowledge of the physical forces that regulate the interactions between various membrane components and their response to intra- and extracellular ligands such as $Ca^{++}$, ATP and acetylcholine. Spin label studies of planar lipid multibilayers indicate that some phospholipids can form orientated lamellar structure. Cholesterol is shown to regulate the order and rigidity of the bilayer structure of some phospholipids but not others. The latter results suggest the presence of specific lipid-lipid interactions and it is conceivable that certain lipid(s) could be localized at the membrane surface. These localized lipids can serve as the receptor for protein or ligand binding sites by providing specific hydrophobic and charge interactions. The intermolecular charge-charge interaction affects the order and the phase of certain phospholipids. The neutralization of the charge-charge interaction with electrolytes, pH or proteins also affects the phase, organization and stability of the phospholipid bilayer structure. The dynamic aspects of membrane structure can be studied by measuring the transverse motional freedom of spin labeled lipids in viable biological membranes. Recently we have demonstrated that it is possible to measure the rate of inward translocation of a membrane bound myristicamide-spin label in intact erythrocytes. The level of intracellular ATP and $Ca^{++}$ concentrations controls the rate of inward translocation of the probe by regulating the fluidity of the intact erythrocyte membrane. This lecture summarizes the potential applications of the spin labeling method and some significant new information on biological membrane structure and dynamics.

## 105

THE NATURE OF MEMBRANEOUS FRACTIONS DERIVED FROM YEAST PROTOPLASTS. J.B.M. RATTRAY, A. SCHIBECI and D.K. KIDBY, Dept. of Chemistry, University of Guelph, Guelph, Ont., Canada.

The plasma membrane is implicated in several important physiological functions of the yeast cell, but little information is available on the basis for its biological activity in terms of chemical composition and structure. Consideration has been given to methods routinely available for the preparation of yeast plasma membrane and an assessment made of their effectiveness in yielding material of well defined purity. A method involving ultracentrifugation on a discontinuous sucrose density gradient has been applied successfully to a lysed protoplast preparation derived from *Saccharomyces cerevisiae*. Definite subcellular entities have been obtained and identified on the basis of centrifugal behavior, composition analysis and from phase-contrast and electron microscopy. The application of enzyme marked determination has been found to be generally unsatisfactory. In addition to a plasma membrane fraction, other membraneous systems have been derived from "microsomes," vacuoles and nuclei. The protein to phospholipid ratio has been determined to vary between the fractions. The distribution of $^{32}P$ between the various membraneous systems, obtained on growing yeast cells in the presence of $^{32}P$-orthophosphate has been measured. Thin layer chromatographic analysis of chloroform-methanol extracts of the several subcellular fractions has revealed the major, but variable, presence of phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine and phosphatidyl inositol. Certain unidentified phospholipids, sphingolipids and glycolipids have been determined to possess a minor occurrence. The yield of more polar phospholipids has not been found to be enhanced on extraction with acid chloroform-methanol. Considerable variation in the distribution of free and esterified sterol, free fatty acid and triglyceride has been observed. It has been possible from the results obtained to make a comparison of the general lipid composition of the whole cell and the various subcellular fractions of yeast.

## 106

MITOCHONDRIAL AUTONOMY IN THE BIOSYNTHESIS OF POLYGLYCEROPHOSPHATIDES. N.Z. STANACEV, Dept. of Pathological Chemistry, Faculty of Medicine, Banting Institute, University of Toronto, Toronto 181, Ont., Canada.

Cardiolipin (diphosphatidylglycerol) is the most characteristic phospholipid of mitochondria accumulated on the inner mitochondrial membrane and is one of the major phospholipids found in mitochondria. Recent reports from several laboratories have established that mitochondria cannot synthesize lecithins and cephalins and therefore depend on the endoplasmic reticulum for these lipids, where they are synthesized and then translocated to mitochondria. Can mitochondria synthesize cardiolipin and if so, by which mechanism, is the subject of this presentation based on the experimental results obtained in this laboratory. Two possible pathways for the biosynthesis of cardiolipin from phosphatidylglycerol can be represented by reaction (1) and (2). 2 Phosphatidylglycerol → Cardiolipin + Glycerol.....(1); Phosphatidylglycerol + CDP-diglyceride → Cardiolipin + CMP.....(2). Phosphatidylglycerol involved in both pathways can be synthesized according to the following sequence of reactions: *sn*-Glycero-3-phosphate + 2 Acyl-CoA → Phosphatidic acid.....(3); Phosphatidic acid + CTP → CDP-diglyceride.....(4); CDP-diglyceride + *sn*-Glycero-3-phosphate → Phosphatidylglycerophosphate.....(5); Phosphatidylglycerophosphate → Phosphatidylglycerol.....(6). Experimental results evaluating mitochondrial ability to synthesize phosphatidic acid, phosphatidylglycerol and cardiolipin will be presented in detail. These results have established that mitochondria cannot synthesize phosphatidic acid by the acylation of *sn*-glycero-3-phosphate, since the biosynthesis of phosphatidic acid by this pathway, often in the literature assigned to mitochondria, should instead be attributed to the microsomal contamination of mitochondria. Mitochondria, however, can synthesize phosphatidylglycerol; indeed this synthesis appears to be an exclusively mitochondrial process.

Incorporation of phosphatidylglycerol into cardiolipin is also a mitochondrial process. In addition detailed experimental evidence establishing the participation of both phosphatidylglycerol and CDP-diglyceride in the formation of cardiolipin according to reaction (2) is presented. These findings have established that mitochondria possess the necessary enzymatic machinery for the formation of phosphatidylglycerol and cardiolipin, although they are dependent on the endoplasmic reticulum for the formation of some necessary precursors and for other phospholipids.

## 107

FAT MELTING POINT DETERMINATIONS: A REVIEW. W.G. MERTENS, Canada Packers Ltd., 2211 St. Clair Avenue West, Toronto 167, Ont., Canada.

The determination of melting points is a frequent and routine analytical procedure for most industrial oil laboratories. In its relation to the body temperature, the melting point can be a good indication of the "mouth feel" of a product and of its behavior at elevated room temperatures. Yet in a true sense one cannot speak of "the melting point" of a natural fat, since these complex glyceride mixtures do not have a distinct, sharp melting point but a melting range. Fat crystals can exist in several polymorphic modifications, which makes the temperature-time pretreatment of the sample important. For all melting point methods, specific test conditions have therefore been established, and one point within the total melting range is selected arbitrarily as the melting point. Changes in the established procedures can produce variations in the results, and different methods usually give results not directly related to each other. A number of melting point methods require subjective interpretations of the end point, other methods use easily noticeable physical changes. Newer methods, which could promise a degree of automation, time saving, or an improvement in the precision of the results, will always be of interest. Some experience with two instruments, for a micro melting point and for an automatic dropping point, will be discussed briefly.

## 108

FAT POLYMORPHISM. E.S. LUTTON, 5656 Ridge Ave., Cincinnati, Ohio 45213.

The story of glyceride polymorphism is long and troubled with controversy, but major issues relating to major glycerides have been settled by coordinated use of X-ray diffraction and thermal measurement including differential thermal analysis (DTA), with an assist from IR spectroscopy. While the picture is complex, an appreciative acquaintance with the $\alpha$, $\beta$ and $\beta'$ double chain length structures of tristearin is an adequate basis for dealing with many problems. In understanding the broad features, it is helpful to relate glyceride polymorphism to that of other long chain compounds. A considerable understanding of polymorphism and phase behavior, in general, is necessary for adequate control of shortening, margarine and enrobing fat behavior, especially when a significant degree of composition variation is encountered. There are still academic and practical aspects of glyceride crystal polymorphism which deserve further exploration. In any case, complete consideration of fat polymorphism (or liquid crystal formation) is not to be ignored, especially in cases involving aqueous phospholipids, monoglycerides or other emulsifiers.

## 109

DIELECTRIC BEHAVIOR OF TRIPALMITIN AND TRISTEARIN. C.W. HOERR and F.R. PAULICKA, Glidden-Durkee Div. of SCM Corp., P.O. Box 8827, Strongsville, Ohio 44136.

The dielectric constants of tripalmitin and tristearin have been measured over a wide range of temperature. Data are presented for the liquid state and for the lowest and highest melting forms of the solids. Interpretation of the dielectric behavior provides further understanding of the polymorphic transformations of these compounds. In conjunction with X-ray diffraction and polarized-light microscopy, the dielectric data afford a new insight into the influence of tempering on crystal structure. In addition to the well known effect of tempering in bringing about polymorphic transformations, tempering also alters molecular orientation by more efficient packing in the crystal lattice with a consequent increase in melting point without further transformation to another crystal form. On the basis of the data collected, an accurate phase diagram is established for binary mixtures of tripalmitin and tristearin. This confirms that the system exists as a continuous series of solid solutions with a minimum melting mixture containing 27% tristearin.

## 110

RHEOLOGICAL PROPERTIES OF HARD BUTTERS. F.R. PAULICKA and T.J. BANSEK, Glidden-Durkee Div. of SCM Corp., P.O. Box 8827, Strongsville, Ohio 44136.

Results of an investigation of the relative hardness of domestic and lauric-derived hard butters commonly employed in the formulation of confectionery coatings are presented. The method employed to measure this empirical mechanical property is discussed. Influences of composition, previous thermal treatments, polymorphism, test conditions, etc., on hardness are demonstrated. Hardness is correlated with solid fat index.

## 111

DETERMINATION OF THE SOLIDS CONTENT IN PARTLY CRYSTALLIZED FATS. ADOLF J. HAIGHTON, Unilever Research Laboratory, Olivier Van Noortlaan 120, Vlaardingen, Holland.

A review will be given of the main methods proposed for the measurement of the SFI of fats, viz., dilatometry, calorimetry, differential scanning calorimetry and the NMR techniques (wide-line and pulsed). These methods can give accurate results and are comparable, provided the fat is given the same temperature treatment. Hence emphasis will be laid on tempering procedures and their influence on the results. Causes of differences are mostly due to different degrees of supercooling, polymorphism and formation of different solid solutions. The work carried out for the Instrumental Techniques Committee by the NMR subcommittee will be reported. The use of SFI figures in a factory for quality control, hydrogenation control and prediction of spreadability will be dealt with.

## 112

A COMPARISON OF THE WIDELINE AND PULSED NMR METHODS FOR DETERMINING SOLID-LIQUID RATIOS IN FATS. GARETH J. TEMPLEMAN and ENG C. WANG, The Pillsbury Co., 311 Second St., SE, Minneapolis, Minn. 55414.

Measurements of the solid-liquid ratio of blends of safflower oil and 5.4 IV tallow tempered at five temperatures are reported. The solids in fat index is calculated from the free induction decay of the $^1H$ NMR signal following a single 90° pulse using a commercially available process analyzer. A method of controlling the temperature in the sample probe is described. The results compare with those previously obtained on the same samples by a wideline NMR method. In order to obtain consistent results it is necessary at each temperature to carefully tune the r.f. transmitter and calibrate the instrument, making corrections for magnetic field inhomogeneity.

## 113

COMPARATIVE STUDY OF THE EUTECTIC BODIED OIL WITH STAND OIL BY NMR SPECTRAL ANALYSES. S.N. KOLEY, Dept. of Applied Chemistry 92, A charya Prafulla Ch. Rd., Calcutta 9, India.

The advent of a nuclear magnetic resonance spectrophotometer opens a new horizon for the analysis of organic compounds. Here an attempt is made to apply NMR techniques for a comparative study of eutectic bodied oil with stand oil. The linseed oil was bodied to viscosities ranging from 1.21–4.35 stokes. The eutectic bodied oil was prepared by passing through a saltbath consisting of 54.5% $KNO_3$ and 45.5% $NaNO_2$ at temperatures varying from 280–320 C. The apparatus used was Varian A60A Model Spectrophotometer. The bodied oil and its esters were fractionated using acetone, urea, vacuum distillation and column chromatographic techniques. Unlike the stand oil, the eutectic bodied oil is heterogeneous in nature and separates into two layers on standing. The analyses by NMR show the presence of aromatic and ether compounds in eutectic bodied oil only (1.6 protons present in dimer). The aromatic compounds may be formed by the disproportionation of cyclohexinic protons. The etherial protons may be formed by the interaction of hydroxyl and oxirane protons. The diminuation of diallydic protons is much higher in eutectic bodied oil than the stand oil (diallylic protons reduced to traces in the "oxi" products). The diminuation of olefinic protons varies from 1.5–2.5 in both the cases. The diminuation of $\alpha$-methylentic protons is slight in both the cases. In case of stand oil, the results show that the diels-alder condensation is not the sole reaction; polymerization may also take place through free-radical mechanism.

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THERMAL ALTERATION OF FATTY ACIDS AND THEIR DERIVATIVES IN THE PRESENCE OF MINERAL CATALYSTS. A. EISNER, T.A. FOGLIA and I. SCHMELTZ, E. Market. Nutr. Res. Div., ARS. USDA, 600 East Mermaid Lane, Philadelphia, Pa. 19118.

Fatty acids and their esters were subjected to varying thermal treatments to determine the extent of isomerization and possible inter- or intramolecular condensation. When methyl tallowate, for example, was heated under reduced pressure and in the absence of catalysts, the product showed little change from the starting material. The addition of 1% of sulfur as a catalyst under the same conditions induced some elaidinization but only minor changes in the gas liquid chromatographic (GLC) pattern. Bleaching clay exerted a similar effect. Significant changes were observed when the methyl tallowate was heated, with 5% of clay added as a catalyst, in a sealed glass tube for 24 hr at 290 C. Similar effects were obtained with methyl oleate under the same conditions and with oleic acid at 240 C. The changes were evident by comparing the GLC pattern of the modified product with that of the starting material, especially in the $C_{18}$ region. Iodine value determinations of the products show that a large part of the original unsaturation was retained. Less than the theoretical amount of hydrogen was absorbed when the products were subjected to low pressure (50 psi) catalytic hydrogenation. The results suggest a strong possibility of isomerization or branching of the carbon chain, or both.

## 115

CATALYTIC HYDROCARBOXYLATION OF FATS. POLYCARBOXY ACIDS FROM MONO- AND POLYUNSATURATES. E.N. FRANKEL and F.L. THOMAS.

A highly selective catalytic, one-step synthesis converts oleic acid into 9(10)-carboxystearic acid in high yields (85–99%). Hydrocarboxylation with water and carbon monoxide under pressure (3000–4000 psi) is catalyzed with a mixture of palladium chloride and triphenylphosphine at 140–150 C with or without acetone solvent. Palladium supported on carbon is also an effective hydrocarboxylation catalyst in the presence of triphenylphosphine and HCl. The carboxystearic acid, after purification by molecular distillation, consisted of equal proportion of the 9 and 10 isomers (thin layer chromatography and mass spectrometry). This catalytic hydrocarboxylation procedure is a more efficient route to carboxystearic acid than the two-step hydroformylation-oxidation process reported previously. Catalytic hydrocarboxylation of linseed oil, its fatty acids and those of safflower oil produced a mixture of mono-, di- and tricarboxylated acids. Carboxylated acids, esters and other derivatives have potential industrial applications.

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POLY(AMIDE-ACETALS) AND POLY(ESTER-ACETALS) FROM PENTAERYTHRITOL ACETALS OF METHYL 9(10)-FORMYLSTEARATE. CROSSLINKED STATIONARY PHASES FOR GAS CHROMATOGRAPHY. J.C. COWAN, W.E. NEFF, R.A. AWL and E.H. PRYDE, N. Reg. Res. Lab., 1815 N. University, Peoria, Ill. 61604.

The pentaerythritol acetals of alkyl formylalkanoates like methyl azelaaldehydate and formylstearate are diesters that can be converted to linear polymers by reaction with diols or diamines. Such polymers have latent crosslinking capabilities

at the acetal linkage. Poly(ester-acetals) from methyl azelaaldehydate crosslinked and bonded to acidic, diatomaceous supports give thermally stable, polar, stationary phases for gas chromatography. The azelaaldehydate poly(amide-acetal), which should have a higher degree of thermal stability than the poly(ester-acetal), lacked solubility for ready preparation of column packings. We now report that poly(amide-acetals) as well as poly(ester-acetals) from the pentaerythritol acetals of methyl 9(10)-formylstearate (derived from methyl oleate via a selective oxo reaction) are soluble in common solvents and can be used to obtain crosslinked, polymeric stationary phases bonded to the support surface. Crosslinked polymers from the formylstearate diester-acetal and ethylene glycol, ethylene diamine or hexamethylene diamine were prepared and characterized. The crosslinked stationary phases had no phase discontinuity between —50 C and their decomposition temperatures of 300–330 C, and they are potentially useful over this range. Practically, however, their utility is somewhat limited in single column application because of a small but detectable phase bleed between 200–290 C. The packings are useful to 290 C in dual column, compensated systems. Determination of McReynolds' constants showed that the poly(amide-acetal) packing has intermediate polarity and that the poly(ester-acetal) packing is relatively nonpolar. With temperature programing these packings resolved, for example, a mixture of 2-pentanone, hexanal, heptanal, 1-hexanol, 1-heptanol, dimethyl glutarate, nonanal dimethyl acetal, 1-nonanol, 1-decanol, butyl nonanoate, hexadecane, methyl 8-formyloctanoate and methyl tetradecanoate. Under isothermal conditions, these sorbents separated a mixture of benzene, 1,4-dioxane, 1-nitropropane, pyridine and *cis*-hydrindane.

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NMR CHEMICAL SHIFT REAGENTS IN STRUCTURAL DETERMINATION OF LIPID DERIVATIVES. IV: METHYL 9,10-DIHYDROXY- AND EPOXYSTEARATE. JOHN P. WINEBURG and DANIEL SWERN, Fels Research Institute, Dept. of Chemistry, Temple University, Philadelphia, Pa. 19122.

Additional structural information can be obtained in $^1H$ nuclear magnetic resonance studies of lipid derivatives when they contain functional groups that can be complexed by chemical shift reagents (csr). The amount of additional structural information that can be obtained is limited to protons that are a maximum of eight carbons away from a csr coordination site. Unsaturated lipid derivatives, however, are potentially capable of providing additional data by derivatization. Although carbon-carbon double bonds do not have sufficient Lewis basicity to complex, they can be derivatized to afford additional sites for complexation. The feasibility of performing csr studies of polyfunctional lipid derivatives was demonstrated in our latest report (JOCS-AOCS 1972 Joint Meeeting, Los Angeles, Calif.). This paper will describe Eu(fod)$_3$ studies of the following methyl oleate derivatives: methyl *erythro-* and *threo*-9,10-dihydroxystearates and methyl *cis-* and *trans*-9,10-epoxystearates. Structural analysis of the major portions of these molecules is now possible. However decoupling experiments are necessary in order to assure correct proton assignments.

## 118

THE EFFECTS OF AMMONIATION ON AFLATOXINS IN RATIONS FED LACTATING COWS. JOHN D. MCKINNEY and GEORGE C. CAVANAGH, Ranchers Cotton Oil, P.O. Box 248, Fresno, Calif. 93708.

Abstract not available at press time.

## 119

HEAT INACTIVATION OF TRYPSIN INHIBITOR AND SOYBEAN ENZYME—THE EFFECT OF ACID AND BASE ADDITIVES. E.C. BAKER and G.C. MUSTAKAS, N. Reg. Res. Lab., 1815 North University St., Peoria, Ill. 61604.

Combining chemical additives in the cooking processes represents a simple and economical approach to improve the food quality of soybeans. Their nutritional value increases when growth-inhibiting factors such as trypsin inhibitor, are inactivated. Inactivation of lipoxygenase enhances palatability and storage stability. First, heat inactivation of soybean enzymes during immersion cooking was studied without additives. Next, hydrochloric acid or sodium hydroxide was added to the cooking water at concentrations of 0.1–1.0%. Cooking from 15 min to 2 hr was evaluated over a temperature range of 120–212 F. Without additives, lipoxygenase proved to be the most heat labile and trypsin inhibitor, the least. With both acid and base additives, enzyme inactivation accelerated significantly; however trypsin inhibitor inactivation was accelerated by base but retarded by acid addition.

## 120

CREAMING IN EVAPORATED MILK. K. SABHARWAL and P.M.T. HANSEN, Dept. of Food Science and Nutrition, The Ohio State University, Columbus, Ohio 43210.

Creaming behavior of evaporated milk as affected by different levels of carrageenan and phosphate was investigated. Triplicate samples of product manufactured on different dates were collected from a commercial plant. Each can of evaporated milk was thoroughly mixed and then stored quiescently at 20 C. After 1 month, the cans were opened and the milk was evaluated for cream line, grain formation and serum separation at the bottom, and chemically for fat and protein at different locations in the can. In addition the electrical conductivity of the milk was determined and selected samples were subjected to electron microscopic examination. The results suggested that creaming of evaporated milk is associated with faulty fat dispersion and incomplete fat-protein interaction during manufacturing. The degree of creaming was significantly related to the electrical conductivity of the milk possibly as a result of the various additions of phosphate. Also addition of small amounts of carrageenan was effective in decreasing the extent of creaming on storage. It was concluded that industry needs more refined methods to determine the types and the practical levels of usage of stabilizing additives for manufacturing of evaporated milk of uniformly high quality.

## 121

DETECTION OF SULFATED CEREBROSIDES IN *GLOMERELLA CINGULATA*. GREGORY E. ANEKWE and LINDA L. LEE, Dept. of Chemistry, Tuskegee Institute, Tuskegee Institute, Ala. 36088.

Sulfated cerebrosides have been discovered in the mycelia of the fungus *Glomerella cingulata*. One liter quantities of chemically defined nutrient medium were inoculated with 60 mg *G. cingulata* spores. The medium also contained 1 ml trace elements solution and 0.8 mC of $Na_2^{35}SO_4$. The material was incubated with shaking and 2- and 3-day-old mycelial samples were collected and total lipids extracted from them. Sulfated cerebrosides were obtained by elution with chloroform, chloroform-methanol 4:1 v/v and chloroform-methanol 9:1, respectively. Preliminary identification was made by thin layer chromatography, using appropriate sulfolipid standards. The fractions were further localized by radioautography. Average period of exposure of the thin layer plates to X-ray films was 3 weeks. The specific radioactivities of the sulfated cerebrosides were also determined. Preliminary studies on the suspected sulfated cerebrosides suggested that they are hexosyl-sulfolipids. Attempts are now being made to identify their fatty acid moieties by analytical gas liquid chromatography. Because *G. cingulata* is an achlorophyllous plant pathogen, we suggested that the sulfated cerebroside fractions may be involved in some other membrane-related activities than photosynthesis.

## 122

CHANGES IN THE STRUCTURE OF TRIGLYCERIDES FROM MATURING KERNELS OF CORN (*Zea mays L.*). EVELYN J. WEBER, ARS, USDA, 230 Davenport Hall, University of Illinois, Urbana, Ill. 61801.

Kernels of the corn inbred, H51, were collected at five intervals after hand pollination. The triglycerides were extracted with petroleum ether and isolated by silicic acid thin layer chromatography (TLC). The most active period of triglyceride synthesis occurred from 20–45 days after pollination when the weight of triglycerides per kernel increased from 1.1–7.5 mg. Over all the collection periods the percentages of palmitic, linoleic and linolenic acids decreased while oleic acid increased, but from 30–60 days after pollination the fatty acid composition of the triglycerides was nearly constant. Stereospecific analysis of the total triglycerides revealed a general fatty acid pattern for the triglycerides where the concentration of the saturated acids was highest in position 1, linoleic acid in 2 and oleic acid in 3. From 20–60 days after pollination there was little change in the fatty acid composition at the 1 position, but the largest changes occurred at the 3 position where palmitic and oleic acids decreased 5.1% and 7.3%, respectively, and linoleic acid increased 13.4%. The variations in the molecular species of the triglycerides were determined by silver nitrate TLC and were found to be small from 20–60 days after pollination except for an increase in trilinolein from 5.2–11.9%. Stereospecific analyses of four major triglyceride species, SMD, MsD, SD$_2$ and MD$_2$, revealed larger changes in fatty acid distribution at individual positions during maturation than were apparent from analyses of the total triglycerides.

## 123

BIOSYNTHESIS OF PHOSPHOLIPIDS IN CELL-FREE EXTRACTS OF SPINACH LEAVES. M.O. MARSHALL and MORRIS KATES, Dept. of Biochemistry, University of Ottawa, Ottawa, Ont., K1N 6N5 Canada.

Biosynthesis of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol has been investigated in cell-free extracts of spinach leaves. Phosphatidyl inositol appears to be synthesized de novo by the CDP-diglyceride + inositol pathway, but evidence indicating synthesis by exchange reactions has also been obtained. Phosphatidyl choline can be synthesized by the established diglyceride + CDP-choline pathway and also by transmethylation of phosphatidyl ethanolamine with S-adenosylmethionine. Studies on the pathways for synthesis of phosphatidyl ethanolamine and phosphatidyl serine will be described.

## 124

FATTY ACID 12- AND 15-DESATURATION BY *PENICILLIUM CHRYSOGONUM* Q176. ROBERTA L. RICHARDS and FORREST W. QUACKENBUSH, Dept. of Biochemistry, Purdue University, Lafayette, Ind. 47907.

*Penicillium chrysogenum* Q176, grown in agitated liquid media, produces an abundance of polyunsaturated lipid. About 70% of the fatty acids isolated from this lipid consists of linoleic (18:2) and linolenic (18:3) acids as shown by gas chromatography of their methyl esters. The relative amounts of these two unsaturated fatty acids vary with age of the culture and this is taken as evidence for two different desaturases, i.e., a 12-desaturase that forms linoleate from oleate and a 15-desaturase that forms linolenate from linoleate. The factors that control the two enzyme systems and determine their relative activity are being investigated. In a culture grown from a spore suspension, concentration of both 18:2 and 18:3 rise rapidly during the first 16 hr and reach maxima at ca. 60% and 12% of the total acids, respectively, by 24 hr. Thereafter, 18:3 falls precipitously while 18:2 plateaus or may increase very slowly. The fall of 18:3 coincides with a marked drop in pH of the medium and a pronounced clumping of the mycelia. It is not prevented by addition of CaCO$_3$ to the medium, increased nitrate in the medium or by passing O$_2$ through the medium. It is postponed by dispersion (Virtis homogenization) of the mycelia at 24 hr. Attempts to effect dispersion by adding various surface active agents have not succeeded. Glycerol and triacetin tend to keep the mycelia dispersed and to reduce the density of clumps which form, thereby perhaps promoting diffusion of O$_2$ to the desaturation site. Although glycerol enhances polyunsaturate formation, triacetin sharply reduces desaturation. Disintegration of the mycelia by sonic bursts in the cold yields a homogenate that retains part of its oleate desaturating activity. After centrifugation at 15,000 × g, the majority of this activity remains in the supernatant fraction. Efforts are directed toward further fractionation of the cell-free extract with respect to the two desaturases.

## 125

THE ROLE OF MEMBRANE LIPIDS IN LOW TEMPERATURE ADAPTATION OF PLANTS. DARYL G. RICHARDSON and CONRAD J. WEISER, Laboratory of Plant

Hardiness, Dept. of Horticultural Science, University of Minnesota, St. Paul, Minn. 55912.

Changes in membrane lipid fatty acyl moieties are being studied during acclimation to low temperatures using various plant species grown under natural and controlled environmental conditions. Cabbage, garden peas, winter and spring wheat, dogwood, and three varieties of apple differing in cold acclimation ability show the same trends toward increased unsaturation of fatty acyl groups accompanying adaptation to low temperatures. It is postulated that adaptation to resist low (and high) temperatures is reflected in the proper control of membrane fluidity by altering the amounts of unsaturated fatty acids esterified in the phospholipids. This would allow the membrane to retain functional semipermeability even at very low temperatures and permit cellular water to freeze in the intercellular spaces instead of within the cytoplasm. Intracellular freezing is almost invariably lethal. Because subcellular organelles exhibit marked differences in lipid class compositions and the distribution of acyl group unsaturation is nonrandom among lipid classes, the organelles themselves should also exhibit differences in ability to withstand low temperature stress. While many researchers have asserted that freezing damage occurs at the *membrane* level, few have attempted to isolate membranes or to characterize the type of injury occurring in the membrane. Phase changes have been shown to occur within both artificial lipid bilayers and intact cells in response to both low and high temperature stress. Damage could conceivably occur at these transition temperatures resulting in destruction of membrane integrity or by exposing critical subcellular areas to degradative enzymes, some of which may have appreciable activity at temperatures as low as −40 C. Recent studies in other laboratories indicate that lipid peroxidation may be a prime factor in injury to animal hepatocytes.

## 126

LEAF WAX OF *PORTULACA OLERACEA* L. A.P. TULLOCH, National Research Council of Canada, Prairie Reg. Lab., Saskatoon, Sask., Canada.

*Portulace oleracea,* which is a troublesome annual weed widespread in North America, can remain alive for long periods after being uprooted. The composition of the leaf wax was therefore investigated. Though the plant does not appear waxy, a normal quantity of wax was isolated. The wax differs from many other plant waxes in having long chain esters as major constituents. The esters are $C_{40}$-$C_{56}$ compounds with $C_{46}$ and $C_{48}$ major components and are apparently formed by random esterification of $C_{22}$-$C_{28}$ alcohols (major component $C_{24}$) by $C_{20}$-$C_{36}$ acids (major component $C_{22}$). Hydrocarbons, $C_{27}$-$C_{35}$ ($C_{33}$ major component) were lesser constituents of the wax and free alcohols ($C_{22}$-$C_{28}$) and free acids were very minor constituents.

## 127

STEREOSPECIFIC ANALYSIS OF TRIACYLGLYCEROLS FROM LIPOMYCES LIPOFERUS. JAMES E. HALBY and R. CECIL JACK, Dept. of Biology, St. John's University, Jamaica, N.Y. 11432.

The stereospecific distribution of fatty acids from triacylglycerols of the yeast *Lipomyces lipoferus* was determined. The triacylglycerols, which represent 70% of the total lipids of the yeast, were isolated by silicic acid column chromatography. The isolated triacylglycerols were checked for purity by thin layer chromatography and were subjected to two reactions: to enzymatic hydrolysis with pancreatic lipase and to Grignard deacylation. The 2-monoacylglycerols recovered from the enzymatic hydrolysis were used to determine the distribution of fatty acids at position 2. The diacylglycerols from the enzymatic hydrolysis and Grignard deacylation were isolated by thin layer chromatography and were converted to 1,2-diacyl-*sn*-glycero-3-phosphorylphenols and 1,3-diacyl-*sn*-glycero-2-phosphorylphenols. These phosphatidylphenols were then subjected to enzymatic hydrolysis with phospholipase A to obtain lysophosphatidylphenols. Fatty acid methyl esters, prepared from the lysophosphatidylphenols, were analyzed by gas liquid chromatography to give the distribution of fatty acids at positions 1 and 3. It was found that the fatty acids at position 2 were predominantly unsaturated and predominantly 18 carbons or longer. Oleic acid was the predominant fatty acid at position 2. Relatively large proportions of C16 and shorter chain saturated fatty acids were found at positions 1 and 3; however position 1 contained a larger proportion of such saturated fatty acids than position 3.

## 128

PHOSPHOLIPIDS OF *ARTHRODERMA UNCINATUM*. ZOLTAN KISH and R. CECIL JACK, St. John's University, Dept. of Biology, Jamaica, N.Y. 11432.

The phospholipid composition of a mutant strain of the fungus *Arthroderma uncinatum* was compared with that of the wild type. Total lipids were purified on sephadex and separated into neutral lipids and phospholipids by silicic acid column chromatography. An aliquot of the phospholipids was subjected to alkaline hydrolysis and the resulting fatty acids were methylated and then subjected to gas liquid chromatography on polar and nonpolar liquid phases. The phospholipids which remained after removal of the aliquot for gas liquid chromatography were separated into classes by DEAE cellulose column chromatography. The identities of the fractions obtained from this separation were checked by two procedures: by thin layer chromatography and by deacylation of the phospholipids followed by paper chromatography of the water-soluble phosphate diesters. The amounts of each class of phospholipid were quantitated by determining the phosphorus content of the fractions from DEAE cellulose chromatography. It was found that phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, diphosphatidylglycerol and phosphatidylinositol were present and that there was marked variation in the amounts of phosphatidylcholine (PC) and phosphatidylserine (PS) in the two strains. The ratio of PC (wild type) to PC (mutant) was 2:1; the ratio of PS (wild type) to PS (mutant) was 1:3. Both the mutant and the wild type contained C16 and C18 fatty acids, but there was significant variation in the proportions C18 unsaturated fatty acids in the two strains.

## 129

LIPID COMPOSITION OF MORNING GLORY CELL SUSPENSION CULTURES (*IPOMEA SP.*). MOONJA SONG and NEIL TATTRIE, Div. of Biological Sciences, National Research Council of Canada, Ottawa, Ont. K1A OR6, Canada.

Recent studies have suggested that plant cell suspension cultures could be a useful system to study lipid biosynthesis. The changes of *Ipomea Sp.* lipids were observed during various stages of growth of the cells. Rapid increases in lipid content were observed 24 hr after the medium was inoculated until 72 hr where the active growth of cells began. Free sterols ($\beta$-sitosterol, stigmasterol and campesterol) were the most abundant class of lipids during the 6 day growth period. Fatty acids of the various classes of lipids were determined by gas liquid chromatography (GLC). Using Na-1-$C^{14}$ acetate the maximum incorporation into the total lipids was between 24 and 48 hr. Specific activities of major phospholipids and sterols were determined. Over 65% of the total radioactivity incorporated into the neutral lipid was recovered in the free sterol fraction during the entire growth period.

## 130

OXYGENATION OF UNSATURATED FATTY ACIDS IN SEEDS DURING STORAGE. G.F. SPENCER, F.R. EARLE and W.H. TALLENT, N. Market. Nutr. Res. Div., ARS, USDA, 1815 N. University St., Peoria, Ill. 61604, and I.A. WOLFF.

Seeds of *Cichorium intybus* L., *Crepis thomsonii* Babc. and *Crepis vesicaria* L. were stored for 4–8 years at 5 C and then for 18 months under a variety of conditions. Oxygenated acids in *Cichorium intybus* oil increased from Ca. 1% initially to 3% in the first storage period and to 17% during storage at room temperature in the second period. The corresponding levels at these three stages for *Crepis thomsonii* were 2, 6 and 18%. By gas chromatography and gas chromatography-mass spectrometry, the major oxygenated acids formed during storage were identified as hydroxy acids with conjugated unsaturation and 9,10-epoxy acids. In *Crepis vesicaria* seed, the oil of which contained 53% vernolic (12,13-epoxy-9-octadecenoic) acid originally, ca. 2% of 9,10-epoxides were formed during the 18 months at room temperature. Levels of hydroxy acids with conjugated unsaturation in this species were 0.3% initially, 2% after 5 years at 5 C and 9% after the 18 months at room temperature. Primary substrates for the formation of oxygenated acids in the three species were crepenynic and linoleic acids, and the almost exclusive formation of 9,10-epoxide from the latter indicated enzymatic involvement.

## 131

INFLUENCE OF DIETARY FATTY ACIDS ON THE LIVER MICROSOMAL FATTY ACID ACTIVATION OF *CIS*- AND *TRANS*-Δ9-OCTADECENOIC ACID IN VITRO. GUSTAV GRAFF and F.A. KUMMEROW, Burnsides Research Lab., University of Illinois, Urbana, Ill. 61801.

Male weanling rats from the Holtzman strain were fed ad libitum three different types of diets: (1) fat deficient, (2) lab chow, and (3) a *trans* fatty acid-containing diet. After 3 months the animals were killed, the livers excised and used for microsomal preparation. The activation of elaidic acid and oleic acid was studied using a system consisting of microsomal protein, ATP, CoA, $MgCl_2$ KF, cysteine and hydroxylamine at a pH of 7.4. The corresponding hydroxamates formed were determined spectrophotometrically in the presence of Hill reagent. The results indicated, that oleic acid had some self-controlling effect on its activation, which was not found for the *trans* isomer. Furthermore differences were found in the rate and energy of activation.

## 132

METABOLISM OF 1,2-DIHYDROXYHEPTADECANE AND 1-HYDROXY-2-KETOHEPTADECANE IN MAMMALIAN BRAIN. H.H.O. SCHMID and T. MURAMATSU, The Hormel Institute, 801 16th Ave. N.E., Austin, Minn. 55912.

Long chain [2-$^{14}$C] labeled 1,2-dihydroxyheptadecane and 1-hydroxy-2-ketoheptadecane were administered intracerebrally to weanling rats. Incorporation of radioactivity from both precursors into the neutral lipids and into the ethanolamine and choline phosphatides of brain was determined after 6 hr. Major radioactive products from either precursor were 1-*O*-2′-hydroxyheptadecyl-2-acyl ethanolamine and choline phosphatides as well as the corresponding 1-*O*-2′-ketoheptadecyl-2-acyl glycerophosphatides. Long chain 1,2-diols and ketols were found to be readily interconverted by the brain. The data presented allow the conclusion that glycerol ether formation in mammalian brain is rather nonspecific and can lead to the 2′-hydroxy or 2′-keto analogs. Obviously plasmalogen formation does not involve a dehydration of 2′-hydroxyalkyl derivatives.

## 133

HUMAN PERIPHERAL NERVE MYELIN: QUALITATIVE AND QUANTITATIVE STUDIES WITH AGING. HARBHAJAN SINGH, NORTON SPRITZ and BARBARA GEYER, Dept. of Medicine, New York University School of Medicine, and Lipid Metabolism Lab., Veterans Administration Hospital, New York, N.Y. 10010.

The entire intraabdominal portions of femoral nerves were obtained from 14 people who died suddenly. After removal of collagen following treatment with glycine buffer (Nature 220:171 [1968]), myelin (purity established by chloroform-methanol solubility and EM) was isolated quantitatively and purified in a cesium chloride continuous gradient. In eight people aged 23–42, myelin content averaged 7.3 ± 2.0 mgm/gm of nerve compared to six aged 60–75 in whom the average was 3.2 ± 1.6 ($p < .001$). This striking difference was evident whether myelin content was related to whole nerve segment or unit nerve length, rather than to nerve weight, indicating that the lower values in the older group reflected an absolute rather than a relative decrease. Total nerve protein, cholesterol and glycolipid did not appear to vary significantly with age. The density (ultracentrifugal flotation) and *composition of myelin* was essentially the same in both groups and resembled that found by O'Brien (J. Neurochem. 14:357 [1967]) in ox spinal roots. Molar ratios of cholesterol phospholipid-glycolipid were 3:3:1; and among the phospholipids, molar ratios of phosphatidyl choline-phosphatidyl ethanolamine-phosphatidal ethanolamine-phosphatidyl serine-sphingomyelin approached 2:1:4:2 5 in both age groups. Protein content was 35.3 and 34.5%, respectively, in the younger and older groups ($p > 0.5$) and Eng-Smith ratios 1.2 and 1.4. Decrease of myelin with aging as revealed by direct measurement may be the counterpart of altered neurophysiology

with aging that is expressed by decreased nerve conduction velocity and loss of vibration sense. Whether the observed decrease reflects a loss of neuronal units or a fall in average myelin content per unit is not established, although the constancy with age of nonmyelin composition supports the latter.

## 134

INHIBITION OF DESATURATION AND ELONGATION OF FATTY ACIDS BY ACETYLENIC ACID. H.C. Chang and Ralph T. Holman, Dept. of Biochemistry, The Hormel Institute, University of Minnesota, 801 16th Ave.. N.E., Austin, Minn. 55912.

Stearolic acid (18 ≡ 1 ω9) was found to inhibit desaturation and elongation of saturated and unsaturated fatty acids by rat liver microsomes. At substrate-inhibitor ratio of 1:1, the desaturations of 18:0 and 18:2 were found to be 70 and 50% of control values, whereas the elongation of 16:0 and 18:2 were about 75 and 80% of control values, respectively. The degree of inhibition varied from preparation to preparation, and depended upon conditions. Several levels of inhibitors were tested with several substrates, and the inhibition curves were similar. Stearolic acid inhibited the incorporation of 18:0 into PL. but had little effect upon incorporation into other lipids. Chain shortening was not significantly inhibited by stearolic acid.

## 135

LONG CHAIN CYCLIC ACETALS OF GLYCEROL. A STRUCTURAL STUDY. Wolfgang J. Baumann, The Hormel Institute, 801 16th Ave. N.E., Austin, Minn. 55912.

Controversy still exists, whether long chain cyclic acetals of glycerol are natural constituents of tissue lipids or rather artifacts produced during hydrolysis of plasmalogen-type compounds. We have therefore studied the formation of the four structural and geometrical isomers of long chain cyclic acetals as they are produced either by condensation of aldehyde and glycerol or by cyclizatiin of 1-alk-1'-enyl-*sn*-glycerol in acidic media. Separation of the isomers was accomplished by adsorption and gas liquid chromatography. The structure of each isomer was established by chemical and spectroscopic methods. Configurations and conformations were determined by NMR spectroscopy aided by deuterium-labeling. The isomers were identified as *cis*-2-alkyl-5-hydroxy-1,3-dioxane, *trans*-2-alkyl-5-hydroxy-1,3-dioxane, *cis*-2-alkyl-4-hydroxymethyl-1,3-dioxolane and *trans*-2-alkyl-4-hydroxymethyl-1,3-dioxolane. Cyclization of alk-1-enyl glycerol ether with *p*-toluenesulfonic acid in boiling benzene led to a thermodynamically equilibrated mixture of acetal isomers in which the *cis*-isomers predominated. Cyclization in acetic acid was found to be kinetically controlled and formation of the *trans*-isomers was relatively favored.

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METABOLISM OF LONG CHAIN CYCLIC ACETALS OF GLYCEROL IN MAMMALIAN BRAIN. K.L. Su, W.J. Baumann, T. Madson and H.H.O. Schmid, The Hormel Institute, 801 16th Ave. N.E., Austin, Minn. 55912.

The four isomeric cyclic glycerol acetals, [$^{14}$C] labeled at carbon-2 of the ring structures, were administered intracerebrally to weanling rats. Incorporation of radioactivity into neutral lipids and glycerophosphatides was determined after 6 hr and 24 hr in order to establish whether cyclic glycerol acetals are acylated, phosphorylated or converted into the common lipid classes of brain, or both. Incorporation of *cis* and *trans* 1,3-dioxolanes (the five-membered ring isomers) appeared to be favored over the 1,3-dioxanes (the six-membered ring isomers). Major amounts of radioactivity were recovered in the acyl moieties of the choline and ethanolamine phosphatides.

## 137

IMPROVED METHODS OF CONVERSION OF 9,10,12,13-DIEPOXYSTEARIC ACID TO 9,10,12,13-TETRAHYDROXYSTEARIC ACIDS. M.A. Khuddus and Daniel Swern, Fels Research Institute, Dept. of Chemistry, Temple University, Phila., Pa. 19122, and Y. Usui.

The conversion of monoepoxides to 1,2-dihydroxy compounds by acid hydrolysis is a well known process which gives good to quantitative yields. Diepoxides, however, in which the oxirane groups are separated by one or two carbon atoms, do not give good yields of tetrols. Previous workers who studied the aqueous acid hydrolysis of diepoxides, such as 1,2,4,5-diepoxypentane, 1,2,5,6-diepoxyhexane, 1,2,5,6-diepoxycyclooctane and 9,10,12,13-diepoxystearic acid, reported that cyclic ethers (5- and/or 6-membered) are the major or exclusive products. Yields of 9,10,12,13-tetrahydroxystearic acid(s) in the range of only 2–15% have been reported on reaction of 9,10,12,13-diepoxystearic acid with acetic or formic acid followed by basic hydrolysis. Previously we described the stereospecific conversion of *cis*- and *trans*-9,10-epoxystearic acids to the *threo*- and *erythro*-9,10-dihydroxystearic acids, respectively, by treatment of the corresponding epoxy acids with dimethyl sulfoxide (DMSO) and trinitrobenzenesulfonic acid (TNBSA) followed by hydrolysis of the resulting alkoxysulfonium salt(s) (Tetrahedron Lett., 411 [1971]). By application of this new method, *anti-cis*-9,10, *cis*-12,13-diepoxystearic acid (A-DESA) is converted into a mixture of *threo*-9,10, *threo*-10,12, *threo*-12,13 (TTT) and *threo*-9,10, *erythro*-10,12, *threo*-12,13 (TET) tetrahydroxystearic acids in 50–60% yields (TTT/TET = 2:1). When A-DESA is treated with 97% formic acid followed by basic hydrolysis, the same two isomers are obtained in 60–68% yields but in a different ratio (TTT/TET = 9:1). Mechanisms of formation, stereochemistry and gas liquid chromatographic analysis of these two isomers will be discussed. The factor(s) that influence cyclization in competition with formation of tetrahydroxystearic acid(s) will also be discussed.

## 138

AN AUTOMATED THERMOGRAVIMETRIC SYSTEM FOR THE MICRODETERMINATION OF LIPIDS. Bipinchandra Patel, J. Leerkamp and A.N. Siakotos, Dept. of Pathology, Indiana University Medical Center, 1100 W. Michigan Street, Indianapolis, Ind. 46202.

The determination of total weights of mixtures of lipids and lipid-like materials has often required chemical analysis for polar components, such as phosphorus, sugars, sulfate; or general reactions unique to the hydrocarbon nature of lipids have been employed, e.g., oxidation of chromate, quantitative densitometry, etc. Although a procedure has been available for the thermogravimetric mass determination of lipid mixtures by applying discrete volumes of dissolved lipids to a microbalance pan, followed by evaporation of the solvent with heat and then cooling until a constant weight was obtained. This procedure required considerable operator skill and time to provide reproducible and true values. These drawbacks have inhibited acceptance of this approach to quantitation of lipid mixtures. Our approach has been to employ a commercial thermogravimetric apparatus (TGA) modified to provide highly reproducible results without constant supervision and negligible operator error. The same constant reproducibility is obtained under a variety of atmospheric conditions and is independent of the skill of the technician. In comparison with a previously published procedure, experimental coefficients of variation at the same concentrations per unit volume are equivalent. Although the variation increased with the decreasing sample size, multiple applications were successful in achieving lower coefficients of variation characteristic even with small samples. Reproducibility was found to be independent of sample volumes and was inversely related to sample weights.

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A STUDY OF THE MINOR CONSTITUENTS IN TALL OIL FATTY ACIDS AND THE IDENTIFICATION OF *Trans*-3,5-DIMETHOXYSTILBENE. David B. S. Min and Stephen S. Chang, Dept. of Food Science, Rutgers State University, P.O. Box 231, New Brunswick, N.J. 08903.

The high quality tall oil fatty acids (1.0% unsaponifiables and 1.0% rosin acids) have a tendency to develop a dark color when heated. In addition, they produce a red color when treated with an expoxidizing solution. The present investigation found that the development of the dark color during heating is due to the presence of minor constituents and that the formation of a red color during epoxidation is due to the presence of *trans*-3,5-dimethoxystilbene. The high quality tall oil fatty acids were passed through a silicic acid column to obtain purified tall oil fatty acids which had none of the defects mentioned. The minor constituents retained on the chromatographic column were separated into ethyl ether-eluted and methanol-eluted compounds. The latter were more detrimental to the color stability of purified tall oil fatty acids than the former. However the amount of methanol eluted minor constituents was less than the ethyl ether eluted products. Each of the two fractions were separated into acidic and nonacidic minor constituents. From the nonacidic ethyl ether eluted minor constituents, a fine white crystal was isolated by low temperature solvent fractional crystallization. This compound was identified as *trans*-3,5-dimethoxystilbene by the combined use of UV, IR, mass and NMR spectrometry. This identified compound was found to be responsible for the development of the red color during epoxidation of the high quality, tall oil fatty acids.

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QUANTITATIVE ANALYSIS OF COMPLEX LIPID MIXTURES WITH A NEW SCANNER FOR THIN LAYER CHROMATOGRAPHY. K.D. Mukherjee and H.K. Mangold, Bundesanstalt für Fettforschung, Institut für Technologie und Biochemie, H.P. Kaufmann-Institut, 44 Münster (Westf.), Germany.

Organic compounds that are separated on layers of adsorbent coated on the internal walls of pyrex or quartz tubes can be detected and quantitatively estimated by an instrument which has been described recently (K.D. Mukherjee et al., J. Chromatogr. 61:317[1971]). The substances in the various solute zones are vaporized consecutively from the adsorbent into a stream of nitrogen, either by pyrolysis, or, if the adsorbent contains cupric oxide, by combustion in situ. The products of pyrolysis are monitored by a flame ionization detector (FID) either directly (Pyrolysis-Detection method, PD), or after their combustion to carbon dioxide and subsequent reduction to methane (Pyrolysis-Combustion-Reduction-Detection method, PCRD). Alternatively carbon dioxide formed by in situ combustion is reduced to methane and detected in the FID (Combustion-Reduction-Detection method, CRD). The solute zones are recorded as peaks by a strip chart recorder; the amount of each fraction in a mixture is determined from the peak areas. The performance of this instrument has been tested using model mixtures of lipids. Errors in quantification are well below ± 5% relative. A wide variety of complex lipid mixtures, including plant lipids, unusual lipids of marine animals and human tissue lipids have been analyzed by this instrument with excellent speed and accuracy. Further applications of this instrument in routine analysis in clinical laboratories and in biochemical studies are outlined.

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ASPECTS OF POROUS POLYMER BEADS FOR QUANTITATIVE ANALYSIS OF VOLATILE FATTY ACIDS AND ANOMALOUS PEAK BROADENING EFFECTS FOR METHYL-BRANCHED MATERIALS. R.G. Ackman, Fisheries Research Board of Canada, Halifax Lab., P.O. Box 429, Halifax, N.S., Canada. The passage of VFA through columns of either Chromosorb 101 Porapak QS in stainless steel, determined with a flame ionization detector, is complete when formic acid is added to the carrier gas. Tailing is notably suppressed. Cyclohexanone provides a very suitable internal standard. Isobutyric acid has an exalted response relative to butyric acid. It was observed that mono- and gem-dimethylbranched acids and ketones had peak widths greater than those for corresponding linear molecules on Porapak QS but none on Chromosorb 101.