ABSTRACTS OF PAPERS

1

ACCOUNT OF DR. LYMAN'S RESEARCH ON GOSSYPOL AND ITS SIGNIFICANCE AND IMPACT ON THE COTTONSEED INDUSTRY. GARLON HARPER, National Cottonseed Products Association, Memphis, Tenn. 38112.

Abstract not available at press time.

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DETERMINATION OF STRUCTURE AND SYNTHESIS OF GOSSYPOL. J. D. EDWARDS, JR., Department of Chemistry, University of Southwestern Louisiana, La.

The reported synthesis of the cottonseed pigment, gossypol, and degradation product of it containing the basic ring structure will be presented and discussed. The preparation from these products of important derivatives will also be given as will the recent isolation of optically active gossypol from a different plant.

3

THE EFFECTS OF DIETARY GOSSYPOL ON ANIMALS. F. H. SMITH and A. J. CLAWSON, North Carolina State University, Raleigh, N.C. 27607.

The effects of dietary gossypol on cats, rabbits, chickens and calves will be briefly discussed. Its effects on rats and swine will be the area stressed. Included will be toxic effects on each species, the isolation of gossypol from porcine liver and its identification, and the distribution of gossypol in various organs of rats and swine. The effects of minerals in overcoming gossypol toxicity, and its depletion from liver tissue of swine will be discussed.

4

PRACTICAL SIGNIFICANCE OF GOSSYPOL IN FEED FORMULATION. K. J. SMITH, National Cottonseed Products Association, Inc., Memphis, Tenn. 38112.

It has been recognized that gossypol has a limiting effect on the usage of cottonseed meal in nonruminant rations. Extensive research has shown, supported by practical experience, that cottonseed meal may be a major oilseed supplemental protein source in nonruminant rations, when care is taken to accept and adjust for gossypol limitations. This presentation will discuss gossypol limitations and will show how these may be minimized and almost eliminated in the formulation of practical nonruminant rations.

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PREPARATION AND ISOLATION OF A GOSSYPOL-PEPTIDE COMPLEX. C. M. CATER and C. M. LYMAN (Deceased, March 1969), Oilseed Products Research Center, College Station, Tex. 77843.

The presence of bound gossypol in cottonseed protein prevents complete digestion and utilization of the constituent amino acids. A procedure for isolating bound gossypol-containing peptides would permit determination of their amino acid composition and provide a basis for supplementation of rations containing this material. A model complex of gossypol and bovine insulin, whose structure and amino acid composition is known, was subjected to enzymic digestion and fractionated by liquid-liquid extraction, gel filtration, ion-exchange chromatography, electrophoresis, and thin layer chromatography. Procedures were developed which permitted the isolation of a relatively pure gossypol-octapeptide complex. Studies revealed that gossypol may participate in exchange reactions during the course of enzymic digestion and may become bonded to peptides other than, or in addition to, its original site. This was demonstrated by the isolation of a gossypol-peptide complex after the addition of gossypol-β-alanine or gossypol-3-aminopropanol complexes to a solution of bovine insulin and incubating with trypsin.

6

DETERMINATION OF GOSSYPOL: PAST AND PRESENT. W. A. PONS, JR., So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

The evaluation of analytical methods for the determination of gossypol and gossypol-like pigments in cottonseed products will be reviewed, with emphasis on the development of the concepts of "free" and "bound" gossypol pigments. The disadvantages of present nonspecific methods for free and bound gossypol pigments and the need for more specific methods for estimating native gossypol and classes of derived gossypol pigments will be discussed.

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THE LIPID CHARACTERISTICS OF HUMAN OMENTAL TISSUE. F. E. LUDDY, S. F. HERB, PHILIP SKEERETT and J. L. RABINOWITZ, E. Utiliz. Res. Dev. Div., ARS, USDA, Philadelphia, Pa. 19118.

Recently, Goldsmith described a new and dramatic surgical technique involving omental transposition for the treatment of chronic lymphedema. It is now projected that the technique will be effective in treatment of many other clinical conditions, particularly peripheral vascular insufficiency. Because of the increasing interest in this tissue, we have studied the lipids of the omental fold in several individuals from samples taken at autopsy. The average lipid concentration of the omentum (40–50% of wet tissue) was less than that of adipose tissue (50–70%). The lipids consisted essentially of triglycerides with less than 2% of other components (steryl esters, free acids, sterols and phospholipids). The fatty acid composition of the glycerides was similar to that of human depot fat except for higher values of linoleic acid. The distribution of the fatty acids among the glyceride molecules was also studied by the pancreatic lipase procedure and glyceride compositions were calculated according to Vander Wal's 1,3-random 2-random distribution theory. The data indicate less tendency for the unsaturated acids to occupy the 2-position of the glyceride than adipose glycerides. In one unusual case, a study was made of the omental lipids from an individual with gross loss of weight over an extended period of time. The concentration of lipid in this tissue was less than 1/20th of the normal yield. The fatty acids contained less than 1% of polyunsaturated acids but much higher values for the saturated and unsaturated hexadecanoic acids. The glyceride distribution pattern was, however, similar to that of the normal omental lipid.

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DIETARY REGULATION OF HEPATIC MICROSOMAL ENZYMES FOR GLYCERIDE SYNTHESIS. R. D. WIEGAND, G. A. RAO and RAYMOND REISER, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Tex. 77843.

Several investigators have reported alteration of fatty acid synthetase activity by dietary manipulations. In order to ascertain whether parallel changes occur in the activities of the microsomal enzymes involved in the production of glycerides, standard conditions for their assay were defined using microsomes isolated from livers of rats maintained on a stock diet. Liver microsomes of rats fasted for three to four days and those which were maintained on a fat free-high carbohydrate diet for one week were then assayed for the esterifying enzymes. Unlike fatty acid synthetase, the esterifying enzyme activities did not decrease under fasting conditions. However, there was an appreciable increase in the specific activities of those enzymes in the livers of rats maintained on the fat free diet. Since it is not likely that there is a renewal of enzymes during starvation, it is probable that under that condition the microsomal esterifying enzymes are stable, but that the stimulation of fat synthesis in animals fed high carbohydrate may be due to the enhanced activities of both the soluble and microsomal enzymes involved in this process.

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FATTY ACID COMPOSITION OF MONKEY MILKS. SUHADI HARDJO and L. M. SMITH, Food Science and Technology, University of California, Davis, Calif. 95616.

Milks from six species of monkeys contained 2.2% to 8.5% total lipids. The fatty acid composition of the several milks, and of a pooled sample of human milks, was determined by gas liquid chromatography. The lipids of the monkey milks were generally similar to each other in composition. The predominant fatty acids (by weight) were capric (7.5% to 14.6%), palmitic (19.4% to 23.3%), oleic (22.4% to 30.3%) and linoleic (13.6% to 15.2%). Small amounts of butyric (0.1% to 1.2%) and caproic (0.5% to 0.8%) acids were present in all the monkey milks. The average amount of myristic acid in monkey milk was less than in human milk (2.8% vs. 6.4%). In contrast, there were greater amounts of caprylic (5.9% vs. 0.5%) and capric (11.0% vs. 1.6%) acids in monkey milk. The fatty acid composition of the lipids from each of the six species of monkeys generally resembled that of human milk more closely than that of bovine milk.

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SEX DIFFERENCES IN PHOSPHOLIPID CONTENT OF SERUM, LIVER AND BROWN FAT FOLLOWING COLD-INDUCED LIPID MOBILIZATION. C. A. OLMSTED, K. J. HARDY and YEA-FHEY KUO, Department of Biological Sciences, LSUNO, New Orleans, La. 70122.

Fasting in the cold increased liver phospholipids (PL) and liver total lipids (TL) in female mice, thus relating liver phospholipid content either to the transport of adipose lipids or to the subsequent mobilization of lipids from the liver. In 24 and 48 hr periods of fasting, male mice did not show a similar change except for an increase in total liver lipids. The males did not mobilize as much lipid from brown fat as females during the first 24 hr period. However, in the second 24 hr, i.e., in 48 hr fasted, cold-exposed males, liver PL and liver TL both increased as in females. Thus, it appears that there is a lag in lipid mobilization in males, whereas females mobilize more lipid only initially. These findings suggest that cold exposure promotes mobilization of adipose tissue lipids before a change in liver lipid mobilization occurs. This was tested further by the administration of adrenalin to cold-exposed and normal temperature mice. Adrenalin decreased serum PL very rapidly but only transiently while liver TL increased to a maximum 6 hr after adrenalin administration. Liver and brown fat PL did not change in the early or later intervals after adrenalin. These findings are discussed in relation to the role of phospholipids in the triglyceride cycle, and specifically in lipid mobilization from the liver during cold stress.

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PHOSPHOLIPID METABOLISM AND TRANSPORT IN TUMOR BEARING MICE. J. R. TERRANOVA, K. J. HARDY and C. A. OLMSTED, Department of Biological Sciences, LSUNO, New Orleans, La. 70122.

Male and female mice bearing B-16 mouse melanoma tumor of various sizes showed hepatomegaly and decreased total lipid (TL) in both liver and brown fat. Phospholipids (PL) increased in these tissues but total cholesterol (TC) was variable. PL and TC as a per cent of TL were increased in all cases. Lipid levels in the melanoma tissues remained relatively constant regardless of tumor size. Some of the changes in brown fat TL, PL and TC were related to tumor size being lower in mice with large tumors than in mice with small or medium-sized tumors. Because a sex difference in these aspects of lipid metabolism was not apparent in the tumor bearing mice, only male mice were used for the studies on phospholipid synthesis and transport. Radiophosphate incorporation into PL and the specific activities of phospholipid phosphorus (PLP) of liver, brown fat, and sera were increased in tumor bearing male mice at 3, 6, 12 and 18 hr after administration of the radiophosphate. These findings are related to the possible transport role of phospholipids in the triglyceride cycle. Since the relative specific activity of serum to liver PLP was greater in tumor bearing mice at each time interval studied, it is concluded that a rapidly growing tumor activates the triglyceride cycle because of the increased demands for substrates and for energy metabolism.

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TUMOR LIPIDS: BIOSYNTHESIS OF PLASMALOGENS. Randall Wood and Kathleen Healy, Oak Ridge Associated Universities, Oak Ridge, Tenn. 37830.

Groups of mice bearing Ehrlich ascites cells were administered 1-^{14}C-hexadecanoyl-CoA, 1-^{14}C-hexadecanol, 1-^{14}C-1-^{3}H-hexadecanol, or 1-^{14}C-1-^{3}H-hexadecanal interperitoneally and the combined cells and fluids were collected from the peritoneal cavity 24 and 48 hr after administration of the substrate. The distribution of radioactivity in the acyl, alkyl and alk-1-enyl moieties of various neutral lipids and phospholipid classes was determined. Distribution of activity in various chain lengths, specific activity measurements, 16:0/18:0 specific activity ratios, and ^{3}H/^{14}C ratios of acyl and ether-linked residues at the two time periods were also determined. Comparison of the data obtained with each of the substrates indicate the origin of plasmalogens and their relation to alkyl glyceryl ethers. Alk-1-enyl acyl phosphoglycerides (*plasmalogens*) appear to be derived from intact alkyl acyl phosphoglycerides by biodehydrogenation. Fatty acids are precursors of long chain alcohols that serve as precursors of alkyl glyceryl ethers. Fatty acids were elongated but were not desaturated to the corresponding monoenoic acids. The interconversions of fatty acids, aldehydes and alcohols were also observed. These data enabled us to modify the pathway proposed earlier for the biosynthesis of lipids containing alkyl glyceryl ethers to include the lipids that contain alk-1-enyl ethers.

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PLASMALOGEN BIOSYNTHESIS IN EHRLICH ASCITES CELLS GROWN IN TISSUE CULTURE. Randall Wood, Marva Walton, Kathleen Healy and R. B. Cumming, Oak Ridge Associated Universities, Oak Ridge, Tenn. 37830.

Ehrlich ascites carcinoma cells (2.0–2.5 × 10^{9} cells) and 1-^{14}C-^{3}H-hexadeconol (13 μc ^{14}C, μ 39 μc/mg, ^{3}H/^{14}C ratio 8.57) were transferred aseptically to approximately 1700 ml of culture medium 199 containing calf serum (10%) and antibiotics. Incubations were carried out at 37 C in a 9 liter roller bottle for 12, 24, 36 and 48 hr then the cells were harvested. The highest percentage of activity was found in the lipids of the cells. The percentage distribution of radioactivity in the acyl, alkyl and alk-1-enyl moieties of various neutral lipids and phosphalipid classes was determined at each time period in addition to specific activities and ^{3}H/^{14}C ratio measurements. At 48 hr phosphatidyl ethanolamine plasmalogens represent more than 50% of the total phosphatidyl ethanolamine activity. The complete analyses of lipids obtained from all time periods have not been finished at this time but the data obtained thus far are compatible with those reported previously. Data obtained from these time studies should allow us to establish the classical precursor-product relation between alkyl and alk-1-enyl ethers. The results establish that the biosynthesis of the ether-linked lipids occurs in the Ehrlich ascites carcinoma cells.

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THE FATTY ACID COMPOSITION OF RAT HEARTS AS INFLUENCED BY AGE AND DIETARY FATTY ACIDS. B. F. Szuhaj and R. L. McCarl, Central Soya, Chicago, Ill. 60639.

The fatty acid composition of the neutral and polar lipid fractions from rat hearts was determined in different aged rats as dietary lipid sources changes. The lipids from the rat hearts and dietary sources were purified on a Sephadex G-25 column and separated into neutral and polar fractions by silicic acid column chromatography. The quantitated neutral and polar lipid fractions were hydrolyzed and methylated with BF_3 in methanol. The fatty acids were then chromatographed on ⅛ in. × 10 ft aluminum column of 15% EGS on 80/100 mesh acid washed Chromosorb W. There were three major fatty acids in the neutral lipid fractions which comprised 72% of the total neutral lipid fatty acids. $C_{18:1}$ was the major fatty acid followed by $C_{16:0}$ and $C_{18:0}$. The same three fatty acids were the major fatty acids in the polar lipid fraction and they comprised 83% of the total polar lipid fatty acids. However, $C_{18:0}$ was the major fatty acid followed by $C_{16:0}$ and $C_{18:1}$. On comparing the dietary fatty acids with the changes in composition of the neutral and polar lipid fractions of the whole rat heart, it appeared that the rat heart was capable of selective accumulation and conversion of the dietary fatty acids or both into the composition that is essential for the rat heart.

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STRUCTURAL ANALYSIS OF TRIGLYCERIDES FROM HUMAN AORTIC PLAQUES. R. M. Caley, R. G. Jensen and R. E. Pitas, University of Connecticut, Storrs, Conn. 06268.

Aortas were graded as to severity of sclerosis by the method of Bötcher and a severely sclerosed aorta was chosen for analysis. Lipid was extracted from the plaques by the Folch procedure prior to isolation of the neutral glycerides by preparative thin layer chromatography. The triglycerides were then subjected to a Brockerhoff stereospecific analysis. The intact triglycerides were also analyzed directly by GLC, after hydrogenation, in order to determine their carbon number distribution. The fatty acids were found to be nonrandomly distributed among the 3-positions in that 16:0 predominated in position 3 and was present in equal amounts in positions 1 and 2. Position 3 contained the highest percentage of 18:0 and position 2 the least. Oleic and linoleic acids were in the highest concentration in position 2 and the least amount of each was found in position 3. It is interesting to note, in comparing these results to those of human adipose tissue, that the percentage of unsaturated acids in the 1-position agree quite closely; however, in adipose tissue approximately 70% of the 2-position acids are unsaturated while in the plaque TG only 57% unsaturates are in this position. In adipose tissue 59% of the 3-position fatty acids are unsaturated whereas in the plaques only 27% of the 3-position fatty acids are unsaturated. The predominating species of Triglyceride had a carbon number of 52. Other carbon numbers observed were 46, 48, 50 and 54. Carbon number 50 was the next most abundant species. A carbon number distribution was calculated from the composition of positions 1, 2 and 3 on a 1-random, 2-random, 3-random basis. The plaque triglycerides did not fit this distribution.

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SEPARATION AND QUANTITATION OF ALKENE AND HYDROXY ALKANE SULFONATES BY THIN LAYER CHROMATOGRAPHY. M. C. Allen and T. T. Martin, Continental Oil Company, Ponca City, Okla. 74601.

In the production of straight chain, detergent range olefin sulfonates it is desirable to have a method for the analysis of the compounds produced. This paper describes a thin layer chromatographic technique for the separation and quantitation of the sodium salts of alkene mono- and disulfonates, and hydroxy alkane mono- and disulfonates. The separation is accomplished on tracked, unactivated sulfate-impregnated Silica Gel G layers. Standard saturated developing tank conditions are employed in conjunction with a developing solvent consisting of chloroform-methanol-sulfuric acid. Visualization of the compounds is based on their suitability for charring by heat and SO_3 fumes. The developed layer is placed on a hot plate under a concave, sandblasted glass lid which has been smeared with fuming sulfuric acid. The combination of heat and SO_3 fumes rapidly chars the compounds without contamination from liquid reagents. The charred compounds are quantitated using a scanning photodensitometer; scanning each track from the solvent front to the spot point. Areas of the resultant peaks are calculated and related to composition either by use of calibration standards or by use of correlation factors between per cent carbon and peak areas. The method is rapid, with as many as 15 samples simultaneously analyzed on a 20 × 20 cm thin layer plate.

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VISUALIZATION AND QUANTITATION OF THIN LAYER CHROMATOGRAMS BY AN IMPROVED CHAR TECHNIQUE. T. T. Martin and M. C. Allen, Continental Oil Company, Ponca City, Okla. 74601.

The technique of spraying thin layer chromatograms with powerful oxidizing agents for the purpose of compound visualization is widely accepted and used by thin layer chromatographers. The oxidants are sprayed on the chromatoplate, heated to effect char, and dried to remove water. Several disadvantages are inherent in the procedure: (a) the layer must be sprayed uniformly for quantitation; (b) the oxidizing agents generally are hygroscopic, making it impossible to retain the plates in a laboratory atmosphere; (c) laboratory equipment and personnel must be protected from the strong oxidants; and (d) the reagent must be pure and droplet formation minimized. This paper describes a char procedure whereby a concave, sandblasted, heat-resistant glass lid is smeared with fuming sulfuric acid. The lid encloses the chromatoplate while it is being heated by a hot plate in an atmosphere of dry pyrosulfuric acid fumes. At no time does liquid acid or the glass surface upon which it is smeared come in contact with the chromatographic layer. All sample components are rapidly charred and made clearly visible against a clean background. The charred layer is free of excess acid, making it nonhygroscopic and permanent. The advantages of conventional char techniques are maintained while eliminating the major disadvantages. Optimization of acid strength, time of exposure, temperature of char and layer thickness yields spots which can subsequently be quantitated by densitometric techniques. The use of this char technique introduces a fourth variable, layer thickness, which can be exploited for simultaneous investigation of components by use of fluorescence spectroscopy. Ultraviolet light can be used on the front or back of the plate to observe fluorescent products which are formed by the incomplete reaction with SO_3. The detection of many nonfluorescing sample components as well as those which already possess natural fluorescence can be enhanced manyfold.

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FATTY ACID METHODOLOGY FOR HEAT ABUSED OILS. A. E. Waltking and Helen Zmachinski, Best Foods Division of CPC International, Inc., Bayonne, N.J. 07002.

Criticisms have been voiced of the methods employed for determining the fatty acid composition of heat abused vegetable oils. In the present study, several different vegetable oils were heated, under standardized conditions, to frying temperatures, under air, for various periods of time and then subjected to analyses for fatty acid composition. The methods employed were: ultraviolet spectrophotometry; gas liquid chromatography employing normalization of the peak areas, direct standards and internal standards; and the enzymic, lipoxidase procedure. The latter procedure is preferred in studies wherein measurement of essential fatty acids is a factor of importance. The present findings confirm that the drop in iodine value is a good approximation of the degree of destruction of polyunsaturated fatty acids in heated oils. In testing liquid nonhydrogenated vegetable oils that had been subjected to the standardized conditions of heat abuse, all of the analytical methods for estimation of fatty acid composition, with the exception of normalization of GLC peak areas, give substantially the same result. With hydrogenated vegetable oils the test methods provide values which differ significantly among themselves. Reasons for such differences were published in earlier reports from our laboratory. Evidence is presented to show that the quantity of material retained on the gas chromatographic column is directly related to polyunsaturate loss and measurement of these materials reflects the magnitude of polymer formation. It is for this reason that normalization of the peak areas, in applying gas liquid chromatography to the assay of heat abused vegetable oils, provides erroneously high values for some of the fatty acids.

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DETERMINATION OF RESIDUAL SOLVENT IN OILSEED MEALS AND FLOURS: III. ISOPROPANOL. S. P. Fore, E. T. Rayner and H. P. Dupuy, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

A relatively simple volatilization procedure is described for the determination of residual isopropanol in oilseed meals and flours. A 1.0 g sample of meal or flour is placed in a 120 ml serum bottle, 200 μl of distilled water containing 0.095 μl of ethanol is added, and the bottle is sealed with a rubber stopper and heated at 110 C for 1 hr. A 2 ml aliquot of the head-space gas is analyzed by gas chromatography using the added ethanol as an internal standard. Values determined by this procedure for laboratory preparations of three cottonseed, two peanut and two fish meals or flours, which had been processed with isopropanol, ranged from 10 to 12,000 ppm and were of the same order of magnitude as values determined by a modification of an azeotropic distillation procedure currently being used for analysis of fish meals. Advantages of the volatilization over the azeotropic distillation procedure are that less operator and gas chromatograph time and less sample are required per analysis.

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A SIMPLE TECHNIQUE FOR DETERMINING THE GLC PROFILE OF VOLATILE COMPOUNDS IN VEGETABLE

OILS. K. T. HARTMAN, L. C. ROSE and R. L. VANDAVEER, Frito-Lay Research Department, Irving, Tex. 75060.

A simple procedure has been developed for the isolation, concentration and gas liquid chromatographic (GLC) detection of the volatile compounds in vegetable oils. The volatile compounds are isolated by bubbling purified helium through a measured quantity of vegetable oil heated to 350 F. These compounds are collected on activated charcoal and then extracted from the charcoal with carbon disulfide containing an internal standard. The distribution of the volatile compounds is determined using a GLC system equipped with a flame ionization detector. A 400-fold concentration of the volatile compounds is achieved with this procedure. The technique provides good reproducibility and has been successfully used for measuring the increase of volatile compounds in vegetable oils during storage and snack food processing.

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DETERMINATION OF PLASMA TOCOPHEROLS BY GAS LIQUID CHROMATOGRAPHY. JOANNA LEHMANN and H. T. SLOVER, Human Nutrition Research Division, ARS, USDA, Beltsville, Md. 20705.

A method will be described for the separation and determination of plasma tocopherols by GLC. Proteins in 0.1 g of plasma were precipitated by adding 0.1 ml of ethanol containing a known amount of 5,7-dimethyltocol which serves as internal standard. Tocopherols were extracted into petroleum ether, purified by TLC, and chromatographed as the trimethylsilyl ethers on 0.5% Apiezon L at 250 C. Values for tocopherol content of seven human plasma samples determined by both GLC and Emmerie-Engel methods will be compared. Recoveries of alpha and gamma tocopherols added to plasma samples averaged 100.7% and 88.4%, respectively. Plasma tocopherol levels of 16 human subjects determined by GLC ranged from 5.4 to 14.8 $\mu g/g$ with an average of 9.3 $\mu g/g$. Only gamma and alpha tocopherols (with ranges of 0.6 to 2.2 $\mu g/g$ and 4.2 to 13.1 $\mu g/g$, respectively) were detected in human samples. Plasma samples from several rats analyzed by this method contained beta tocopherol in addition to alpha and gamma.

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QUANTITATIVE DETERMINATION OF RICININE. G. FULLER and H. C. SMITH, JR., Western Regional Research Laboratory, ARS, USDA, Albany, Calif. 94710.

Along with a number of unusual proteinaceous materials, some of them highly toxic or allergenic, castor plants contain an alkalod, ricinine (1-methyl-3-cyano-4-methoxy-2-pyridone), which has been reported to produce only mild physiological effects. For proposed feeding tests on detoxified and deallergenate castor pomace we required information about how much ricinine as well as other castor components were in animal rations and whether or not ricinine is transmitted to animal tissues. A method is reported which is capable of detecting 1 ppm or less of ricinine in aqueous solution. This method, which uses the fluorescence of ricinine, has been applied to determine ricinine content of castor pomace extract. Methodology has also been devised to remove ricinine from interfering substances found in animal tissues. The methods and results will be reported.

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TRIMETHYLSILYL DERIVATIVES OF POLYOLS AND CARBOXYLIC ACIDS IN AQUEOUS SYSTEMS. L. H. PONDER, American Enka Corporation, Enka, N.C. 28728.

The trimethylsilyl (TMS) derivaties are useful in gas chromatography for compounds of low volatility and compounds which tend to absorb or tail on chromatographic columns. However the rapid reaction of TMS reagents with moisture and the hydrolytic instability of the TMS derivatives is a serious disadvantage with substances which cannot be readily dried because of instability or volatility. Further, elimination of the drying step would be advantageous in quality control work where rapid analyses are desired. Several silylating agents and solvent systems have been studied for toleration of water leading to eventual analysis of dilute aqueous solutions. The completeness of reaction is compared for aqueous and nonaqueous systems and for aqueous systems containing quantities of water miscible liquids used to diminish the interference of water. Aqueous solutions of polyols and acids have been studied—glycerol, diethylene glycol and azelaic acid on a quantitative basis and others qualitatively. Applications are illustrated with specific examples and quantitative data for (a) substances which have close retention times but show widely different retention times for their TMS derivatives and (b) for analysis of polyester fiber and fiber finishes.

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TRINITROBENZENE SULFONIC ACID: A REAGENT FOR THE QUANTITATIVE ANALYSIS OF SPHINGOLIPIDS. A. N. SIAKOTOS, Indiana University Medical Center, Indianapolis, Ind. 46202.

A spectrophotometric procedure has been developed for the determination of microquantities of sphingolipids. The assay system involved includes the transesterification of the fatty acid moiety of the sphingolipid with boron trifluoride in methanol to yield a long chain base containing a free amino group. The sphingosine is then extracted into an organic phase and reacted with aqueous trinitrobenzene sulfonic acid (TNBS) to yield a product with an absorption maximum at 340 mμ. Lipid peroxidation products and silicic acid do not seriously interfere in the 340 region. A wide variety of pure sphingolipids yielded equivalent optical densities per mole of sphingolipid. Sphingolipids assayed included cerebroside, cerebroside sulfate, ganglioside and sphingomyelin. Data will be presented to demonstrate the potential applicability of the method to very small tissue samples.

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DELIBERATE BIODEGRADATION IN A CLOSED SYSTEM COOLING TOWER. E. K. HOLT, Lever Brothers Company, New York, N.Y. 10022.

In June of 1966, a small experimental cooling tower was placed in operation at a 30 gpm circulation rate while being fed with make-up water consisting of the mixed effluents of an edible plant and a fatty acids distillation plant after their settling for about 30 min in a conventional skim basin, followed by subsequent aeration and skimming in a Colloidaire separator. Collaborating on this program was E. G. Paulson of the Calgon Division of Hall Laboratories. To simulate summer conditions, the circulating water was heated to 105 F, thus permitting a 20 F temperature drop through the tower. Almost immediately, some aerobic degradation of BOD and COD was observed, so, at Mr. Paulson's suggestion, feeding of the biota with nitrogen and phosphorus was started. The pH was controlled at neutral. This increased biodegradation about fourfold, the tower levelling off with 6% blow-down relative to the rate of feed with BOD's and COD's substantially equal to, or lower than, their level in this feed. Thus, overall biodegradation of 94% was realized, attributed to: (a) aeration in the tower; (b) high water temperature of 85 F; (c) feeding of nitrogen; (d) feeding of phosphorus, and (e) pH adjustment. Subsequently, a larger tower was used which handled total effluent water from the barometric system of a single edible batch deodorizer. In this case, although water temperature had to be lower in order to maintain a proper vacuum on the deodorizer (74 F instead of 85 F), and other conditions not as favorable had to be dealt with, for a period of two months no blowdown whatever was found to be necessary, the COD and BOD leveling off after the first four weeks at about 1730 and 360 ppm respectively with no indication at all that they would not remain there indefinitely. Further tests on fatty acid plant waste waters have been successful, proving to our satisfaction that both deodorizer and fatty acid plant effluents can be bottled up in a properly designed and operated cooling tower deliberately permitted to go "biological." The obvious benefits are twofold, i.e., water conservation, and the elimination of waste water treatment and disposal.

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TABOOS VS. REALITIES REGARDING ANIMAL AND VEGETABLE OILS AND FATS IN MUNICIPAL SYSTEMS. G. N. MCDERMOTT and L. B. POLKOWSKI, Procter & Gamble Company, Cincinnati, Ohio 45217.

Abstract not available at press time.

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JET AERATION OF WASTE WATER. R. W. WEST, Houdaille Industries, Inc.

Water pollution is the topic of today and the aeration of wastewater for the biological reduction of contaminants is one of the most significant treatment methods. Oxygen dissolved into wastewater is used by the bacteria to stabilize the organic matter and form additional bacteria. Carbon dioxide and water are the products of this activity. Jets have been used for many years to aerate wastewater and supply dissolved oxygen to the liquid. Until recent years, the development of this device for this purpose was done by the consumer rather than by the manufacturer because of the nature of the application and the requirement of a "systems" concept instead of a "component" concept. Penberthy Division of Houdaille Industries has recognized the advantages of the jet aeration method and the technological gap that existed in the application of the jet, and is currently continuing a program of data and product development that was begun several years ago at the University of Iowa. The paper briefly reviews the history of Jet Aeration and relates the development of the method to its current status. Unit performance is discussed to illustrate factors of application and their effect on system performance. Parameters of basin mixing, solids distribution, oxygenation, and floc condition are provided. Systems designs are illustrated to demonstrate cost saving ideas that permit equipment costs of $150 per installed horsepower in many applications. Reliability and maintenance factors are presented.

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RECOVERY OF FATTY ACID LIGHTS FROM STILL VAPORS. C. T. ATWOOD and C. L. WOODY, Lever Brothers Company, New York, N.Y. 10022.

Lever Brothers Company operates a fractionating fatty acids still primarily on coconut oil fatty acids in the plant complex at Hammond, Indiana. The still has two condensers in series to condense the top product but until recently 1% to 2% of fatty acid lights normally passed through the hot well. Here the caproic, caprylic and capric acids dissolved in the warm water and these dissolved acids ultimately found their way to the plant effluent stream where they contributed to the B.O.D. loading. Early in 1969 a Croll-Reynolds Scrub Vactor was placed in operation to remove fatty acids from the vapors leaving the still. A normal scrubber installation could not be employed because of the volatility of the lights, but a modification was devised in which coconut oil low in free fatty acids is sprayed into the scrubber. The coconut oil is removed continuously at such a rate that the free fatty acids content of the oil does not build up to over 10% to 15%. This material then goes to the fat splitter. By this means 80% or more of the fatty acids are removed from the vapor stream with resulting reduction in pollution of plant aqueous effluent and collection of salable fatty acid lights.

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PROGRESS TOWARDS EUTROPHICATION CONTROL. T. E. BRENNER and R. C. BLACK, Soap and Detergent Association, New York, N.Y. 10022.

With growing concern over the eutrophication problem particular attention has been directed to the control of the many nutrients, including phosphorus, which are needed to support algal growth. Since phosphorus is an important constituent of detergent products, the industry has been requested to seek different product formulations which would eliminate this material. In response, the industry is actively seeking a replacement material. However, there are many important considerations which make the development of such a material extremely difficult. The industry is sponsoring research on the basic eutrophication process in an effort to gain more definitive information on the relationship between nutrients and nuisance algal growths. The soap and detergent industry is also participating in a joint industry/government task force on eutrophication. A major objective of this group is the development of an algal assay procedure to determine the algal growth potential of chemical agents. Work is also underway to develop information on the economical removal of phosphorus during waste treatment.

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PERACID AND DERIVATIVES: A REVIEW AND COMPARISON OF PROCESSES FOR COMMERCIAL PRODUCTION. W. F. GOLDSMITH and C. A. GIBSON, Union Carbide Corp., South Charleston, W. Va. 25303.

The various commercial processes for producing organic peracids are discussed. Basically, two methods are used in-

dustrially: (a) oxidation of an aldehyde such as acetaldehyde and (b) equilibration of a mixture of hydrogen peroxide and an organic acid such as formic acid or acetic acid. The relative merits of the alternative processes are reviewed. The second phase of the discussion involves the various commercial epoxidation procedures, including their advantages and disadvantages. Emphasis is given to epoxidized oils such as soybean and linseed and to epoxidized esters of tall oil fatty acid. However, the discussion also includes the production of the more reactive epoxides, e.g., various cycloaliphatic compounds, α-olefin derivatives, styrene oxide, etc. Peracetic acid is now available in a variety of solvents for experimental epoxidation, oxidation, or bleaching purposes. Safety considerations involving storage and handling are reviewed.

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EPOXY OILS FROM PLANT SEEDS. F. R. Earle, No. Utiliz. Res. Dev. Div., ARS, USDA, Peoria, Ill. 61604.

The recent discovery of 9,10-epoxy-*trans*-3,*cis*-12-octadecadienoic acid brings to five the number of natural epoxy acids now known to occur in seed oils. The latest epoxy acid and 15,16-epoxy-*cis*-9,*cis*-12-octadecadienoic acid so far have been found in only one species each and at levels lower than 5% of the oil. Coronaric (9,10-epoxy-*cis*-12-octadecenoic) acid and 9,10-epoxystearic acid have been reported in several seed oils, the first as much as 15% of the oil and the latter in only small amounts. Vernolic (12,13-epoxy-*cis*-9-octadecenoic) acid, which has been identified in numerous oils, is the only epoxy acid known to occur in seed oils at levels above 15%, and it may constitute as much as 75% of the oil. On the basis of data available to date, *Vernonia anthelmintica* appears to have the best potential for commercial production of an epoxy oil. One improved line has been selected, but continued improvement is needed. Formation of epoxy acids in oilseed during storage after harvest has been demonstrated recently, and may be partly responsible for the small amounts of epoxide detected in oils from a wide variety of seeds.

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NEW REACTIONS OF THE OXIRANE GROUP. Daniel Swern, Department of Chemistry, Temple University, Philadelphia, Pa. 19122.

Epoxidized oils and esters have been used commercially for about two decades; their consumption is now of the order of 100 million lb./year, mainly as stabilizer-plasticizers for vinyl chloride polymers. Yet in spite of their low price and ready availability, chemical derivatives of these substances have not achieved similar commercialization. It has long been known that the oxirane group is highly reactive and undergoes a wide variety of ring-opening reactions with a broad range of electrophiles and nucleophiles. During the past 10 years, in particular, new and interesting reactions of the oxirane group have been described that provide new routes to other heterocyclic ring systems and functional groups. After a brief background survey of selected older reactions of the oxirane group, recently published and unpublished reactions from the author's laboratory, as well as reactions published by other groups, will be described.

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SELECTION OF PLASTICIZERS FOR OPTIMIZATION OF LOW TEMPERATURE AND PERMANENCE PROPERTIES OF VINYL SYSTEMS. W. J. Gaffigan and J. W. Harlan, Swift & Company, Oak Brook, Ill. 60521.

Polyvinylchloride films intended for low temperature service normally include in their formulations high levels of secondary plasticizers selected to lower the brittle point and epoxidized oils to provide stabilization. The effect of representative secondary plasticizers and epoxidized oils on the viscoelastic properties in the low temperature range has been determined using the Clash-Berg technique (ASTM D-1043 61-T). The Clash-Berg T_4 temperature is considered to be a more relevant indication of a plasticized film's limit of acceptable drape, hand and flexibility when used as an upholstery, garment, or automotive component than the Clash-Berg T_F, or brittle point, which indicates flex-failure temperature. The secondary plasticizers currently used for enhancement of low temperature properties are esters of dibasic acids. The butyl, hexyl and octyl diesters of adipic, sebacic and azelaic acids each have their advantages and disadvantages in terms of low temperature contribution, rate of solvation, volatility, extractability and cost. The lower molecular weight esters are most effective in lowering T_4 and T_F but have poor permanence properties. Dioctyl sebacate, although expensive, is generally accepted as having the best permanence properties of the group. The epoxidized triglyceride oils have excellent permanence properties but a detrimental effect on low temperature flexibility. Epoxy tallates are not detrimental to low temperature properties but do present problems in terms of volatility, compatibility and resistance to extraction. Specialty epoxidized esters have been evolved which at normal use levels are comparable in low temperature properties to epoxy tallate but have lower volatility and greater resistance to extraction. These specialty epoxidized esters are more effective in enhancing low temperature properties using the Clash-Berg T_4 criteria than dioctyl sebacate at normal use levels and also provide fast fusion times and good solvency when compared to either dioctyl sebacate or epoxy tallates.

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CHRONIC TOXICITY OF VERNONIA SEED OIL IN RATS. B. R. Zeitlin, S. F. Biscardi and M. N. George, General Foods Corp., Tarrytown, N.Y. 10591.

Feeding of Vernonia seed oil containing 70–75% triglyceryl ester of 12,13-epoxyoleic acid at levels of 1%, 2% and 4% to Sprague Dawley rats results in elevation of urine ketones, and mortality at the 4% level. Many animals which died at the 4% level demonstrated hemorrhage of the leptomeninges, bleeding in the thoracic cavity. Histopathology examination demonstrated the target organs to be the brain and heart in animals maintained on 4% diets dying before the 16th month of study. Chronic tracheal inflammation was observed in many of the 4% test group. The majority of animals killed at 2 years are still under study. Fatty acid analysis of epididymal padlipid extracts in animals maintained for 90 days on Vernonia seed oil showed a trend toward relative increase in saturated fat content. The epoxide was found to be stored in the fat at a concentration of 5–6%.

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SELECTIVE HYDROGENATION OF TRIPLE BONDS TO DOUBLE BONDS IN OCTADECADIYNOIC AND SANTALBIC METHYL ESTERS. S. G. Morris and Paul Magidman, E. Utiliz. Res. Dev. Div., ARS, USDA, Philadelphia, Pa. 19118.

The object was to noncatalytically hydrogenate triple bonds in fatty acids to *cis* double bonds with minimum reduction of any double bonds present or produced. The following powdered metals, aluminum, cobalt, chromium and iron, were used separately as reducing agents in varying concentrations of *n*-propyl alcohol and water. The metals were also treated with varying water dilutions of propionic and acetic acids ranging from 5% to concentrated acids. Mossy granular cadmium and magnesium turnings were similarly treated. Nearly all the metals in hydrogenation mixtures yielded the desired product in 45–70% yields, but the diene groups were also hydrogenated to produce too much of the monoenoic species. Powdered iron in 15% *n*-propanol, and also in pure water, converted the $-C\equiv C-C=C-$ group (yne-*trans* ene) of santalbic acid to *cis,trans* diene in 90% yields, and thus consequently low yields of *trans,trans* double bonds and monoenes. Similarly high yields of 70–80% of *cis,cis* diene acids were obtained from the octadecadiynoic acids.

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INFLUENCE OF SOLVENT AND DEGREE OF ACYLATION ON THE FORMATION OF SUCROSE ESTERS. T. J. Weiss, M. L. Brown, H. J. Zeringue, Jr. and R. O. Feuge, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

Sucrose palmitates were prepared by interesterification of sucrose and methyl palmitate. Reactions were conducted using different solvent systems. The ratio of methyl palmitate to sucrose was varied to establish the relative reactivities of the sucrose hydroxyls. Sucrose esters prepared in dimethylformamide (DMF) were observed to follow random distribution patterns for the palmitoyl groups when only penta- and lower esters were formed in appreciable amounts. When the proportion of palmitoyl groups was increased, hexa- through octaesters were formed, but in yields below those calculated for random distribution. The interesterification of sucrose and methyl palmitate in hexamethylphosphoramide (HMPA) under conditions which were expected to produce penta- through octaesters yielded only mono- through tetraesters. HMPA and DMF used under identical conditions yielded different reaction products. Large proportions of hexa- through octaesters could only be produced by the interaction of sucrose and palmitoyl chloride.

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PREPARATION OF EPIMINOSTEARATES: COMPARISON OF METHODS. T. A. Foglia, G. Maerker and G. R. Smith, E. Utiliz. Res. Dev. Div., ARS, USDA, Philadelphia, Pa. 19118.

Epiminostearates, the nitrogen analogs of epoxystearates, are a new class of compounds which have considerable potential as chemical derivatives of fats. Their synthesis, however, is expensive and tedious since it requires the use of pseudohalogen iodine isocyanate, prepared from silver nitrate, potassium cyanate and iodine. In our search for less expensive and more direct methods of synthesis, we have studied the preparation of these fat-derived aziridines by the addition of dichlorourethan (DCU) to *cis*- and *trans*-octadecene, methyl oleate and methyl elaidate, followed by base-catalyzed ring closure. A comparison of yields revealed that with *cis*-olefins the iodine isocyanate (INCO) route gives somewhat greater amounts of purified aziridines than the DCU method, but with *trans*-olefins the INCO procedure gives lower yields than DCU. In order to compare the stereochemistry of the two methods a spectroscopic procedure was developed which permits an estimation of the relative amounts of *cis*- and *trans*-aziridines in mixtures of the two. In agreement with literature reports, the INCO method was found to be stereospecific, the *cis*-olefin giving rise to *cis*-aziridines and the *trans*-olefin forming the *trans*-aziridine. On the other hand, DCU addition to olefins gives mixtures of *cis*- and *trans*-aziridines in which the latter predominates. This finding is compatible with the reported free-radical nature of the DCU reaction.

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CYANOETHYLATION OF METHYL 9(10) KETOSTEARATE. H. E. Kenney, E. T. Donahue and G. Maerker, E. Utiliz. Res. Dev. Div., ARS, USDA, Philadelphia, Pa. 19118.

As part of our continuing study of the derivatization of aliphatic compounds related to fats, the cyanoethylation of long chain ketones was examined in detail. Reaction of methyl 9(10)-ketostearate with acrylonitrile under basic catalysis leads to the formation of new carbon-carbon bonds by the introduction of one to four β-cyanoethyl groups on carbon atoms adjacent to the carbonyl function. Five derivatives are possible, since there are two possible dicyanoethyl derivatives, one symmetrical and the other unsymmetrical. The five cyanoethylated products of 4-heptanone have been isolated and identified. A GLC method has been developed to separate and determine four of the cyanoethylated derivatives of methyl 9(10)-ketostearate, but the fifth, the tetracyanoethylated product, cannot be determined by this procedure. With the aid of this analytical method a study of some of the reaction parameters was undertaken to determine the degree to which yields and product distribution could be influenced by change in reaction conditions. Among the factors studied were amount of catalyst, reagent ratio, reaction temperature, use of solvents and precision. These series of experiments revealed that the degree of cyanoethylation of methyl 9(10)-ketostearate can be varied within wide limits, but that monocyanoethylation cannot be accomplished in realistic yields without the simultaneous formation of polycyanoethylated products.

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DIMER ACID STRUCTURES: CYCLIC STRUCTURES OF CLAY CATALYZED DIMERS OF NORMAL LINOLEIC ACID, 9-CIS,12-CIS-OCTADECADIENOIC ACID. D. H. Wheeler, A. Milun and F. Linn, General Mills, Inc., Minneapolis, Minn. 55413.

The clay catalyzed dimer of linoleic acid has been examined by mass spectrometry of the unhydrogenated, the partially hydrogenated and completely hydrogenated dimer. The results show that monocyclic, bicyclic and tricyclic structures are present. Monocyclic structures predominate, bicyclic structures are also prominent, and tricyclic structures are relatively minor. The monocyclic structure is believed to arise from a Diels-Alder type addition reaction. The bicyclic structure may result from a free radical coupling followed by intramolecular ring closure. The monocyclic structure in the unhydrogenated dimer is apparently a benzene ring with saturated side-chains, to a considerable degree. It probably is formed by hydrogen transfer from the Diels-Alder cyclohexene structure first formed.

Little, if any, of the Diels-Alder dimer structure as such is present. The catalytic linoleate dimer has a relatively greater amount of monocyclic dimer than does the noncatalytic (thermal) dimer made from "normal" (nonconjugated) linoleate, while the thermal dimer of a conjugated *trans-trans* linoleate is exclusively monocyclic. It is suggested that the clay catalyzes conjugation and hence favors the Diels-Alder reaction, and then catalyzes hydrogen transfer to aromatize the cyclohexene ring.

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THE REACTIONS OF NITRATE ESTERS: THE SYNTHESIS OF NITRILES. J. C. WEKELL, L. W. LEHMAN and E. J. GAUGLITZ, JR., BCF Technological Laboratory, Seattle, Wash. 98102.

Methods for the synthesis of unsaturated fatty nitrate esters are described. The present work demonstrates that the nitrate esters easily undergo nucleophilic substitution. For example, the nitrates and the halides were prepared from oleyl alcohol and alcohols derived from menhaden oil, then converted almost quantitatively (greater than 90%) to the nitrile using sodium cyanide in dimethyl sulfoxide. The nitriles prepared from both the nitrates and the halides have identical properties. The use of the halide as a starting material in organic syntheses on a commercial scale has been limited to the saturated compounds since the preparation of the unsaturated halide is either too expensive or destructive of the double bonds. Therefore, in light of this present work, it is suggested that the nitrate ester can serve as an alternative to the halide in many organic reactions.

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SYNTHESIS OF THE COMPLETE SERIES OF ^{14}C-LABELED CIS AND TRANS OCTADECENOIC ACIDS. J. K. G. KRAMER and R. T. HOLMAN, The Hormel Institute, Austin, Minn. 55912.

All *cis* octadecenoic acids have been previously synthesized and attempts have been made to use these in metabolic studies to elucidate the structure essential to biological activity. However, during the investigation it became apparent that these acids should be labeled and studies should be extended to both geometric isomers. The synthesis of the complete series of ^{14}C-labeled *cis* and *trans* isomers of octadecenoic acids is reported.

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HYDROLYSIS OF 2-IMIDAZOLINES. G. BARUA, Mona Industries, Inc., Paterson, N.J. 07524.

A mechanism of hydrolytic fission of the 2-imidazolines under various conditions has been studied. The influence of various substituents on the rates of hydrolysis and kinetic data are reported. The reactions were base catalyzed and lead to the formation of amides through fission of the ring between position 1 and 2 of the cyclic system. Progress of hydrolysis was followed spectrophotometrically. Experimental studies also revealed that the hydrolysis of 2-imidazolines in acidic solution is very slow or nonexistent. Vapor phase chromatographic analysis was also presented to follow the trend of hydrolysis.

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LIPID TRANSPORT MECHANISMS AT CELLULAR INTERFACES. J. F. DANIELLI, Center for Theoretical Biology, State University of New York at Buffalo, Amherst, N.Y. 14226.

Abstract not available at press time.

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ESSENTIAL FATTY ACID DEFICIENCY AND LIPID MODIFICATIONS IN THE CENTRAL NERVOUS SYSTEM. CLAUDIO GALLI, Institute of Pharmacology, Via Vanvitelli, Milan, Italy 20129.

Essential fatty acid deficiency has been reported to modify several biological parameters (growth, metabolic rate, water balance) and to induce functional changes in various tissues (liver, kidney, lungs). The biochemical alterations observed concern the metabolism and distribution pattern of polyunsaturated fatty acids in tissue lipids. W6 acids decrease, W9 trienoic, triene-tetraene ratios and monoenes increase. The changes in brain lipids and fatty acids, reported in the literature, are generally smaller than those observed in other tissues. EFA deficiency initiated in rats prior to birth, and continued for a prolonged period of time, results in decreased brain weight, brain lipid and phospholipid content, especially in male animals, and considerable changes in polyunsaturated fatty acid distribution, especially in the ethanolamine phosphoglyceride fraction. Trienoic (C_{20} and C_{22}) and docosapentaenoic acids are elevated manyfold, whereas arachidonic, docosatetraenoic and docosahexaenoic acids are much decreased. The unsaturation level of the various phospholipids is maintained at constant levels, in spite of the fatty acid distribution changes. Brain lipid analysis, carried out at various time intervals, in EFA deficient animals indicate that: (a) brain weights are already reduced in 10 day old deficient rats, (b) brain phospholipids decrease, especially in the males after three months, and (c) fatty acid distribution changes in the following order: 1) increase in trienes after three days, 2) decrease of tetraenes (10 days), 3) decrease of hexaenes after six months, and 4) after six months there is an increase of pentaenes, which are elevated at birth but usually disappear after three months of age. The induced changes are only partially reversed upon return of animals to the normal diet. Behavioral changes, especially learning and memory deficiencies, are investigated in these animals.

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SERUM LIPID TRANSPORT SYSTEMS: RECENT ADVANCES. ALFREDO LOPEZ-S., LSU Medical School in New Orleans, New Orleans, La. 70112.

Lipids circulating in the plasma are transported in water-soluble form as lipoprotein complexes. Lipoproteins can be classified according to size, density, electrophoretic mobility and protein composition. The ability of β-lipoproteins (LDL and VLDL) to form complexes with different polyanions has been also used as a method for separation and study of serum lipoproteins. Even within classes of lipoproteins closely related otherwise, the amount of different lipids and the ratios to each other and to protein are variable. Two enzymatic systems seem to be at least in part responsible for the different lipid compositions of serum lipoproteins, lipoprotein lipase and lecithin-cholesterol acyltransferase (LCAT). LCAT, which seems to be associated with α-lipoproteins, is responsible for the formation of the bulk of cholesteryl esters in human serum. Changes in amount of activity of this enzyme may explain the observed changes with age and disease of serum cholesteryl ester fatty acids (CEFA). Differences in CEFA pattern are found between newborn and adult animals, including man. The activity of serum LCAT was observed to increase with age in animals and to be markedly decreased in patients with liver cirrhosis. These patients show abnormal serum CEFA patterns and abnormally low proportions of pre-β-(VLDL) and α-(HDL) lipoproteins.

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ROLE OF PHOSPHOLIPIDS IN THE TRIGLYCERIDE CYCLE. C. A. OLMSTED, Department of Biological Sciences, LSUNO, New Orleans, La. 70122.

Lipid mobilization from the liver to extrahepatic sites of utilization and to adipose tissue for storage and recycling via the triglyceride cycle requires de novo synthesis of liver lecithins. The role of liver phospholipid synthesis and plasma phospholipid turnover has been studied under a number of conditions which appear to relate liver lipoprotein formation and release to liver PL synthesis and transport. Conditions which enhance NEFA release from adipose tissue or which inhibit liver PL synthesis prompt liver lipid accumulation. Lipid accumulates in the liver principally as triglyceride when any one of a number of factors required in liver lipoprotein complex formation is blocked, such as by ethionine, or when NEFA release to the plasma is increased, such as in cold acclimatization. The relationship of liver PL to liver lipid transport is shown also following recovery from these conditions. Recovery from liver triglyceride accumulation is accompanied by increased liver PL synthesis, remobilization of liver PL, and increased turnover of plasma phospholipids. Following the initial depression in liver PL synthesis and transport caused by ethionine administration, the relative specific activity (RSA) of phospholipid phosphorus [SA of plasma to liver PLP] is increased. The RSA is increased also in depancreatized dogs during liver lipid accumulation and in tumor bearing mice during increased adipose tissue lipid mobilization.

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NORSOLORINIC ACID FROM A MUTANT STRAIN OF *ASPERGILLUS PARASITICUS*. LOUISE S. LEE, JOAN W. BENNETT, R. E. LUNDIN and L. A. GOLDBLATT, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

An orange-red pigment has been isolated from a mutant strain of a potent aflatoxin producing strain of *Aspergillus parasiticus*. This new metabolite which represents about 1% of the mycelial mass has a molecular weight of 370, molecular formula $C_{20}H_{18}O_7$ and mp 256–257 C. Functional group tests, elemental analyses, nuclear magnetic resonance and high resolution mass spectra of the pigment and of its methylated derivative established it as 2-hexanoyl-1,3,6,8-tetrahydroxyanthraquinone, (norsolorinic acid), a pigment identified previously in the lichen *Solorina crocea* and the mold *A. versicolor*. Comparison of the ultraviolet and infrared spectra of an authentic sample of norsolorinic acid with the spectra of the *A. parasiticus* metabolite showed the two pigments to be identical. The mutant strain produces much less aflatoxin than does the parent.

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THE USE OF MUTATIONS IN AFLATOXIN RESEARCH. JOAN W. BENNETT, So. Utiliz. Res. Dev. Div., ARS, USDA, New Orleans, La. 70119.

Mutants which produce little or no aflatoxin have been isolated from a strain of *Aspergillus parasiticus* which originally produced high amounts of B and G aflatoxins, and from an *A. flavus* strain which produced B aflatoxins. Many of these aflatoxin mutants are also mutant for mycelial pigmentation. One mutant produces large amounts of the red pigment norsolorinic acid, and has proven to be particularly useful in studying the inheritance of production of aflatoxin where large numbers of colonies must be examined. Conditions such as zinc deprivation and high temperature which inhibit the synthesis of the pigment also inhibit aflatoxin production. Therefore the presence or absence of pigment provides a preliminary screening test for aflatoxin production, and large numbers of colonies may be handled with ease.

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AFLATOXINS M_1 AND M_2: PREPARATION AND PURIFICATION. R. D. STUBBLEFIELD, G. M. SHANNON and O. L. SHOTWELL, Northern Regional Research Laboratory, Peoria, Ill. 61604.

Aflatoxin M_1 has been purified on a series of columns, and M_2 has been prepared by hydrogenation. Crude products containing aflatoxins M_1 and M_2, as well as large quantities of aflatoxins B_1 and B_2 obtained by fermentation of rice with *Aspergillus flavus* NRRL 3251, were chromatographed on silicic acid columns. Almost all the B_1 and B_2 were separated from M_1 and M_2. Aflatoxins M_1 and M_2 were eluted together with ethanol-chloroform (5:95 v/v). The combined M_1 and M_2 fraction was mixed with silica gel (0.05–0.2 mm), the solvent was evaporated, and hte mixture was added to a silica gel column as a slurry in hexane. The column was developed with hexane-chloroform (1:1 v/v) and chloroform to remove traces of B_1 and B_2 and with methanol-chloroform (1.5:98.5 v/v) to elute aflatoxins M_1 and M_2. Most of the colored contaminants remained on the column. Free aflatoxin M_1 and a mixture containing M_1 and M_2 were obtained by rechromatography on a silica gel column. Aflatoxin M_1 from this column was crystallized from acetonitrile. Aflatoxin M_2 was prepared by hydrogenation of crystalline M_1 and isolated by chromatography on a silica gel column. Several solvent systems were found that will resolve aflatoxins M_1 and M_2 on thin layer chromatoplates. These solvent systems were used to follow the elution of M_1 and M_2 from the silica gel columns.

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EFFECT OF A SURFACTANT ON THE PRODUCTION OF AFLATOXINS BY ISOLATES OF *ASPERGILLUS FLAVUS* AND *A. PARASITICUS*. A. F. SCHINDLER and ANNETTE NOBLE, F.D.A., Washington, D.C. 20204.

Isolates were grown in flasks containing Wort broth and in Wort broth plus a surfactant (Tween-80). The media and mycelia were examined separately for the production of aflatoxins after 6, 8 and 13 days growth on a rotary shaker. The addition of 1% surfactant caused a significant increase in production of aflatoxins B_1 and G_1 in mycelia and media

by *Aspergillus parasiticus* isolate M-60. With *A. flavus* isolate M-14 the addition of 1% surfactant resulted in a significant increase in production of B_1 and G_1 in the media at 6, 8 and 13 days. However, the mycelia showed a significant increase only at 13 days. The addition of surfactant also caused a highly significant increase in production of aflatoxin M only in the media and at 13 days. The effect of the surfactant may be due to increased cell permeability. A greater number of mycelial pellets of smaller size was also noted as an effect of the surfactant and may relate to increased mycotoxin production due to greater mycelial surface area.

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UPTAKE AND METABOLISM OF CHOLESTEROL IN RELATION TO REPRODUCTION BY FUNGI OF THE FAMILY PYTHIACEAE, J. W. Hendrix, Department of Plant Pathology, University of Kentucky, Lexington, Ky. 40506.

Species of the plant-pathogenic family Pythiaceae require sterols for sexual and zoosporangial reproduction, but not for growth. These fungi are incapable of sterol synthesis. Cholesterol, supplied at the concentration for incipient biological activity (10^{-6} M), was metabolized to at least two compounds by all of the 13 species of *Pythium* and *Phytophthora* studied. One metabolite appeared to be a fatty acid ester; the other was more polar than cholesterol and was unstable on silica gel thin layer plates. Species varied widely in production of both compounds. Uptake of cholesterol by *Pythium periplocum* was maximum after six days incubation, when growth was complete and reproduction just beginning. Production of the polar metabolite was maximum after three days incubation, and the concentration of this compound gradually decreased through 31 days incubation. Although the cholesterol ester was sometimes present in the medium in large amounts, the polar metabolite was recovered only from the mycelium.

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FATTY ACID COMPOSITION OF *PS. ANGULATA, PS. PHASEOLICOLA* AND *PS. TABACI*. Paul Sacco, J. J. McNeill, P. D. Klein and Patricia Szczepanik, Xavier University College of Pharmacy, New Orleans, La. 70125.

Fatty acid spectra of the above three phytopathogenic bacteria have been studied from the chemotaxonomic viewpoint. The organisms were cultured in a defined medium modified with yeast extract and incubated at 30–34 C for periods of 22–188 hr. All organisms were gram stained for type. The chlorogenic activity of *Ps. phaseolicola* and *Ps. tabaci* was established by inoculating vigorously growing *Nicotiana tabacum* var. Schwarr—Hibshman seedlings. Harvested cells were saponified, fatty acids purified, methylated and subjected to GLC, TLC and mass spectrometry. The available data indicate the uniform presence of $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ saturates and $C_{16:1}$, $C_{18:1}$ unsaturates and hydroxy acids (supported by TLC data). No odd numbered carbon branch chains were found but a C_{15} appeared in *Ps. tabaci* in small but significant amount. *Ps. phaseolicola* contained an appreciable amount of C_{17} and *Ps. tabaci*, C_{17} and C_{19} cyclopropanes. Both organisms are active (chlorogenic) exotoxin producers. A definite relationship was confirmed between the unsaturated ($C_{16:1}$, $C_{18:1}$) components and their homologues, i.e., cyclopropane fatty acids increased in older cultures at the expense of the corresponding unsaturate. Two major components with mass peaks of 113 and 153 are suspected to be short chain oxygenated compounds. However, at the moment, it is not certain that the latter represents the molecular ion. The elucidation of these oxygenated and hydroxy acids is continuing.

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CHARACTERIZATION OF SPHINGOLIPIDS *BACTEROIDES RUMINICOLA*. Eugene Iannotti, Thomas Cook and Mark Keeney, Department of Dairy Science, University of Maryland, College Park, Md. 20742.

The sphingolipids of *Bacteroides ruminicola* were isolated by treatment of the polar lipids with milk alkali and chromatographic fractionation. After spectral and elemental analyses, the lipids were acid hydrolyzed. The water soluble portions indicated ceramide phosphoryl ethanolamine, ceramide phosphoryl glycerol, ceramide phosphoryl serine and unknown compounds. The fatty acids contained branched and straight chains of 15 to 19 carbons. The long chain sphingolipid bases were dihydro-analogues with branched and straight chains of 14 to 20 carbons.

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MINOR LIPID COMPONENTS OF *CLOSTRIDIUM BUTYRICUM*. P. O. Hagen and M. L. Blank, Oak Ridge Associated Universities Medical Division, Oak Ridge, Tenn. 37830.

Compositional studies were made on some of the minor lipid components of *Clostridium butyricum* after reduction of the neutral and polar lipids by $LiA1H_4$. Other than the expected alcohols and O-alk-1-enyl glycerols, material was found which had an R_f on TLC identical with O-alkyl glycerols. Isolation of this material and analysis of its isopropylidine derivatives by TLC showed the presence of three bands. Two of these showed chromatographic characteristics on TLC and GLC identical with standard isopropylidine derivatives of O-alkyl glycerol and long chain alkane-1,2-diol. The components of the third band are at present unknown. It is not known what original polar lipid structure gave rise to these two components. Speculations will be given as to their possible role in the synthesis of *Clostridium butyricum* lipids.

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SELECTION OF HYDROGEN SYSTEMS. D. A. Tanner and J. R. Goodwin, Howe-Baker Engineers, Inc., Tyler, Tex.

An economic study has been made to compare alternatives for the supply of Hydrogen in the range of 50,000 SCFD to 3,000,000 SCFD. All common forms of hydrogen generation were compared. This information will be useful in the selection of generating systems. It points out areas that affect the cost of production, i.e, operating pressure, hydrogen purity, flexibility, maintenance, operator requirements and plant utilization.

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ELECTROLYTIC HYDROGEN GENERATORS—NEW DEVELOPMENTS. A. K. Stuart, The Electrolyser Corp. Ltd., Toronto, Canada.

Present trends in the design of electrolytic hydrogen plants are discussed, with particular reference to packaged plant. The principal types of cell are briefly reviewed, together with rectifier power supply and gas compression and storage systems. Some of the advantages and disadvantages of the electrolytic process are put forward, together with suggestions on how the inherent flexibility of the equipment can be utilized to reduce hydrogen cost under particular industrial situations and to provide for future expansion of capacity.

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NEW DEVELOPMENTS IN HYDROGEN GAS GENERATION-MOLECULAR SIEVES. M. H. Priddy, Surface Combustion Division, Toledo, Ohio 43601.

This paper will cover: (a) Review of conventional processes for producing natural gas steam reformed hydrogen and problem areas associated with complex amine and caustic chemical absorbing systems used for intermediate purification, removal of CO_2. (b) History of molecular sieves—a synthetic dry desiccant material, and the development of a commercial adsorber system for removal of CO_2 and water vapor from process gas streams; (c) Discussion of initial application of the molecular sieve type purifier in nitrogen gas generators and its more recent use with hydrogen producing facilities. (A series of slides containing schematic diagrams and equipment photographs will be used for illustration.). (d) Capabilities of the molecular sieve type hydrogen gas generator related to purity and typical operating cost: typical product gas composition: CO_2, 0.001%; CO, 0.001%; O_2, 0.001%; CH_4, 0.3% to 0.5%; N_2, as present in fuel supply; and H_2, balance. Typical operating cost: nat. gas at 50¢/MCF × 42 CF (reaction), $0.021; × 40 CF (heating), 0.02; water at 6¢/M gal. × 14 gal. (5% tower makeup), 0.001; steam at 75¢/M no. × 47 no. (reaction), 0.035; and electric power at 1½¢/KW × 1 KWH (heating), 0.015. The cost/100 SCF of H_2 is $0.092. The comparative advantages using molecular sieve purification versus chemical absorption in improved gas quality, more simplified and automatic operation, and aspects of reduced maintenance considerations will be outlined.

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THERMAL LIQUID HEATING. D. E. Roush, Monsanto Co., St. Louis, Mo. 63166.

This paper discusses the use of high temperature liquid phase heating medium for industrial, and commercial heating and cooling. A listing of the many types of thermal liquids available to industry for heating and cooling is presented. Particular emphasis is given to the advantages and disadvantages of the various thermal liquids with discussion of thermal limits and properties. Heating system design is of key importance for successful application of thermal liquids. Discussion treats of the entire system as opposed to specific components and sufficient detail and examples are presented to allow an understanding of the basic system. Many new and innovative applications use thermal liquids and these are discussed and illustrated. The paper will be presented with visual aids.

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HIGH TEMPERATURE HEAT TRANSFER EQUIPMENT. E. E. Magnuson, Eclipse Boiler Division, Chattanooga, Tenn. 37405.

The prime purpose of this paper is to outline various alternative methods of high temperature heat transfer in the range of 300 F to 750 F, such as direct firing, forced circulation of heat transfer fluids such as water, oil, Therminol, Dowtherm and other fluids. Advantages and disadvantages of each will be discussed. Important considerations such as the design of heaters, temperature uniformity, heat transfer rates, safety precautions, hardware required, control sequences, fluid degradation, velocities, typical applications, etc., will be discussed.

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A NEW BROAD SPECTRUM SOAP GERMICIDE SYSTEM. Eric Jungermann and David Taber, Armour-Dial, Inc., Chicago, Ill. 60680.

A broad spectrum antibacterial soap is described which utilizes a new germicidal agent: 2-hydroxy-2',4,4'-trichlorodiphenyl ether. It represents the first reported soap germicide system which, in addition to exhibiting activity against a large number of gram positive bacteria, also is active against gram negative bacteria. This paper will discuss the chemical properties of the new system, and will present in vitro and in vivo antibacterial data, efficacy information and a review of safety data.

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POLARITY INDEX—A RAPID MEANS OF CLASSIFYING SURFACTANTS. L. D. Metcalfe, Armour Industrial Chemical Company, McCook, Ill. 60525.

The HLB (Hydrophilic-Lipophilic Balance) system of classifying surfactants is a useful guide for surfactant applications. It has been applied to problems of emulsification, solubilization, wetting, etc. The HLB number can be estimated by several long and tedious procedures. Furthermore, any one of the methods can lead to considerable error. This is particularly true for many fatty nitrogen compounds, new surfactant chemicals and chemicals of unknown chemical structure. It was decided to look at paper chromatography as a rapid means of obtaining similar information on surfactants. The chromatography of these compounds would reflect the total physical and chemical properties that determine surface activity. These properties include solubility, polarity, chain length, surface activity, chemical structure, and functional groups present. A simple paper chromatographic system for assigning a "Polarity Index" number to surfactant chemicals was devised. The sample is spotted on four strips of Whatman No. 1 chromatographic paper. Then four test tube chromatographs are developed in water, ethanol solvent systems (1:0) (7.5:2.5) (0.6:9.4) (0:1). After the chromatographs are developed the spots are visualized by exposure to iodine fumes. The R_f values of the sample are added together and multiplied by 100. The number obtained is called the "Polarity Index" (PI) and can be any number from 0–400. The approximate HLB number can be obtained by dividing the PI by 20. This relatively simple procedure can be used by sales people or other workers in the field with a simple kit. The number obtained can be used in emulsifier selection and other typical surfactant uses.

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COLD WATER DETERGENCY STUDIES USING RADIO-LABELED SOILS. J. C. Illman, B. M. Finger, W. T.

SHEBS and T. B. ALBIN, Shell Development Company, Emeryville, Calif. 94608.

The effects of single and multiple washing and of resoiling/rewashing of cotton and synthetic fabrics have been studied in Tergotometer tests at various levels of temperature, detergent concentration, and water hardness. The soiling mixture consisted of a 7 component sebum tagged with tritium and carbon 14; in some tests gamma-ray emitting Kaolinite clay was also used. Linear primary alcohol ethoxylate (LAEO) and linear alkyl benzene sulfonate (LAS) were used for surfactant type comparisons. In single wash tests in both hot and cold water, LAEO was generally more effective than LAS in removing sebum. This was particularly noticeable at low product concentration where insufficient sodium tripolyphosphate was present to sequester the water hardness. A 1/1 blend of the two surfactants approached LAEO in performance. The nonpolar sebum fraction was more readily removed from Dacron or nylon in cold water; otherwise, detergency was generally poorer at low temperature. In rewash tests, using labeled lube oil, cholesterol and clay, a progressive increase in soil removal was found during five wash cycles. The nonpolar lube oil component was the most difficult to remove from permanent press Dacron/cotton (PP), but was more readily removed from cotton. The more polar cholesterol and especially the clay were more easily removed from PP. LAEO gave better detergency both hot and cold than LAS, especially in hard water. On cotton swatches resoiled with sebum after each wash the residual sebum content was still increasing after five cycles. With PP in soft water, a steady state was reached after 3–5 cycles. Soil buildup was greater as hardness increased and as wash temperature and active matter concentration decreased, and was generally greater on cotton than on PP. LAEO allowed appreciably less soil buildup than did LAS especially at low concentration in hard water, indicating a reduced requirement for sodium tripolyphosphate.

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MICROAEROPHILIC BIODEGRADATION OF TALLOW-BASED ANIONIC DETERGENTS IN RIVER WATER. E. W. MAURER, T. C. CORDON and A. J. STIRTON, E. Utiliz. Res. Dev. Div., ARS, USDA, Philadelphia, Pa. 19118.

Completely anaerobic conditions for biodegradation are seldom realized but are approximated by relatively common microaerophilic conditions under which biodegradation occurs at a low oxygen level of about 1 ppm or less. Tallow-derived alcohol sulfates, ether alcohol sulfates, sulfated alkanolamides, and α-sulfo esters were examined for biodegradability under aerobic and microaerophilic conditions at 25 and 35 C. A petroleum derived linear alkylbenzenesulfonate was used as a reference standard. The tests were carried out in 3 liter aspirator bottles containing 5 ppm or 10 ppm of detergent in 2 liters of river water. Aerobic and microaerophilic experiments were stirred by a continuous stream of air or nitrogen, respectively. The course of biodegradation was followed by analysis for methylene blue active substance using an autoanalyzer. Tallow-based alcohol sulfates and sulfated alkanolamides were the most easily degraded under either condition. Linear alkylbenzenesulfonate was the most resistant.

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SOIL REMOVAL IN RELATION TO TOTAL WORK INPUT: CALORIMETRIC INVESTIGATIONS IN FULL-SIZE WASHERS. LEO LOEB and ROBERT SHUCK, General Electric Company, Louisville, Ky. 40225.

A simple, straightforward method, utilizing the concepts of classical adiabatic calorimetry, for the direct measurement of total energy delivery to a functional wash system is described. For this work a full-sized vertical axis automatic washer was modified for utilization as a calorimeter. The application of this equipment, and the rationale of this approach in studying the energetics of soil removal is discussed. In order to illustrate this technique, the effects of agitation frequency and detergent concentration on power consumption by the wash system are considered.

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BIODEGRADABLE LINEAR ALCOHOL SURFACTANTS FOR USE IN TEXTILE WET PROCESSING. H. T. ZIKA, Union Carbide Corporation, South Charleston, W. Va. 25303.

Highly biodegradable linear alcohol surfactants have proven to be efficient wetting and scouring agents for use in all phases of textile wet processing. The linear alcohol surface active agents, both nonionic and anionic, can be directly substituted for the conventional alkylphenol-based materials, which have been shown to be resistant to biodegradation. No loss in performance or handling characteristics are encountered by changing the surfactant hydrophobe from alkylphenol to linear secondary alcohol. Surfactants based on the linear primary alcohols, which are widely used in biodegradable household detergents, are somewhat less desirable for use with textiles due to generally higher solidification points and less efficient wetting ability. Speciality surfactants, such as low-foaming nonionics or the versatile phosphates can readily be synthesized from the biodegradable linear alcohol hydrophobes. These surfactants are also equivalent to their nonbiodegradable counterparts in textile applications.

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BINDING CHARACTERISTICS OF ANIONIC CELLULOSES. J. B. LAWTON, P. J. BAUGH and G. O. PHILLIPS, Department of Chemistry, University of Salford, Salford M5 4WT, Lancashire, England.

The introduction of carboxymethyl, phosphate and sulphate groups into cotton cellulose considerably modifies the capacity of the cellulose to bind cationic systems. The ion-binding characteristics of these anionic derivatives have been quantitatively studied using the cationic dye methylene blue. Diffuse reflectance spectral measurements demonstrate that two types of sulphated and phosphorylated celluloses can be produced, which differ considerably in the interaction of their anionic groups with the dye. Anionic systems are, in contrast, repelled by the anionic substituents. In this way the resistance of the cotton cellulose to wet soiling can be considerably increased. The mechanism of ion binding to anionic celluloses has been examined and the information utilised to predict methods of modifying the surface characteristics of cotton cellulose.

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RECENT INVESTIGATIONS ON DRUG INTERACTION WITH LIPID TRANSPORT. RODOLFO PAOLETTI and CESARE SIRTORI, Institute of Pharmacology, University of Milan, Via Vanvitelli 32, 20129 Milan, Italy.

The interaction of drugs inhibiting triglyceride hydrolysis and free fatty acid release from adipose tissue (nicotinic acid, antiadrenergic agents, prostaglandins) with the adenylcyclase-phosphodiesterase system in the adipose cells is evaluated. The sensitivity of the hormone sensitive lipase is compared in different experimental conditions and the increased sensitivity to adrenergic stimulation is investigated in fasting and after treatment with diets deficient in essential fatty acid. In both cases the role of prostaglandins is decreased or abolished and therefore the magnitude and significance of the antilipolytic role of prostaglandins at physiological and pharmacological concentrations may be established. The effect shown by a dietary component (essential fatty acids) in modulating the lipase response to adrenergic stimuli and inhibiting drugs and its practical significance are discussed.

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THE FUNCTIONING OF THE LIPIDS AND LIPOPROTEINS OF SARCOTUBULAR MEMBRANES IN CALCIUM TRANSPORT. E. J. MASORO and BYUNG PAL YU, Woman's Medical College of Pennsylvania, Philadelphia, Pa. 19129.

In the intact muscle cell, an internal tubular membrane system called the sarcoplasmic reticulum (SR) plays an important role in the contraction-relaxation cycle by controlling the $[Ca^{++}]$ of the myoplasm; release of Ca^{++} from the SR to myoplasm initiates contractile activity and sequestering Ca^{++} in the SR by means of a transport system causes muscle to relax. Fragments of the SR with a vesicular structure can be isolated from muscle homogenate and these vesicles are able to vigorously transport Ca^{++} from incubation media into the intravesicular space thus enabling study of Ca^{++} transport under precisely defined in vitro conditions. A highly purified fraction of SR vesicles called SF_1 were prepared from rat muscle by means of density gradient centrifugation procedures. The role of SR lipid in Ca^{++} transport was studied. SF_1 was treated in vitro with either phospholipase A or C or D or polyene antibiotics. The effect of essential fatty acid deficiency, induced in vivo, was also investigated. From these studies it was concluded that the only structural feature of SF_1-lipid involved in Ca^{++} transport and the associated ATPase is the phosphoryl moiety of the phospholipids. Evidence was obtained which implicated histidine residues of the SF_1 protein in this transport function. To more deeply probe the role of SF_1 protein in this process, the membranes were solubilized by a sodium dodecylsulfate system and made free of their lipid components. More than 95% of this protein is soluble in dilute salt solution; of this more than 90% is composed of a protein fraction which can be isolated by gel filtration (called protein fraction-2). Protein fraction-2 is a large molecular weight aggregate of identical or similar polypeptide subunits. These subunits have a molecular weight of ~6500, contain solely N-terminal glycine and probably only C-terminal alanine. The significance of such a high percentage of identical or similar polypeptide subunits in SR will be discussed.

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CONTROL OF IMMUNOLOGICAL PHENOMENA BY INTRAVENOUS LIPIDS. N. R. DI LUZIO, Tulane University School of Medicine, New Orleans, La. 70112.

Control of various stages of the immune response has assumed major importance in the areas of transplantation, host-tumor balance, bacterial and viral infections as well as autoimmune disorders. The control of the phagocytic activity of the macrophage cells, which constitutes the afferent limb of the immune reflex arc, has been accomplished in experimental animals by the employment of intravenously administered lipids. Employing the methyl ester of palmitic acid, profound suppression of phagocytic activity by liver and spleen macrophages can be induced. This event is associated with an impairment in the vascular clearance of particulate antigens and a profound depression in both the primary and secondary immune response. Transplantation of skin and tumor homografts is also achievable during methyl palmitate induced depression. Host rejection of the tumor was associated with recovery from depression. Other methyl, ethyl and N-butyl esters of fatty acids containing 14–20 carbon atoms and of various degrees of unsaturation as well as lecithin and cephalin also induced depression of phagocytosis. In marked contrast, the glyceryl ester of oleic acid induced stimulation. Intravenous methyl palmitate also significantly inhibited induction and mortality in endotoxin shock, while enhancing traumatic shock mortality. While the mechanism of impaired macrophage activity and immunological responsiveness following the administration of esterified fatty acids is not as yet clarified, it is apparent that the employment of metabolizable lipid emulsions to control various immunological events offers attractive possibilities in experimental and clinical medicine.

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EFFECT OF EFA DEFICIENCY ON ACCUMULATION AND RELEASE OF TRIGLYCERIDES IN PERFUSED RAT LIVER. TOSHIO FUKAZAWA, O. S. PRIVETT and YOSHIYATA TAKAHASHI, The Hormel Institute, Austin, Minn. 55912.

Studies are reported on accumulation and release of triglycerides in livers of Sprague-Dawley male rats fed a fat-free diet or diets containing hydrogenated coconut oil or corn oil via the technique of isolated perfusion. Perfusions were carried out with Krebs-Ringer bicarbonate buffer containing albumin with and without infusion of oleate or linoleate. Perfusates containing sodium oleate gave an accumulation of triglycerides (TG) in the livers of the corn oil fed animals and stimulated the release of TG into the perfusing medium. In similar experiments with essential fatty acid (EFA) deficient animals (fat-free or hydrogenated coconut oil fed) there was a greater accumulation of triglycerides in the livers and no stimulation in the secretion of triglycerides into the perfusate. Similar results were obtained with linoleate and tracer experiments with 1-^{14}C-oleate and 1-^{14}C-linoleate. Triglycerides of the perfused livers of the animals of the fat-free group in contrast to those of the corn oil group contained relatively large amounts of palmitate and palmitoleate indicating that an EFA deficiency increased the rate of de novo synthesis of fatty acids. It is concluded that fatty livers observed in an EFA deficiency are caused by a number of factors of which one of the most important is impairment in the secretion of triglycerides.

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BUILT-IN LUBRICATION IN COTTON FABRICS. RUTH R. BENERITO, J. B. MCKELVEY and R. J. BERNI, Southern Regional Research Lab., New Orleans, La. 70119.

Partial cotton esters of long chain fatty acids of low degree of substitution [degree of substitution (DS) <0.15] have been prepared by a variety of methods. In particular, the effects of the introduction of a stearoyl or an oleoyl group into the cellulose matrix on fabric properties such as lubricity, flex abrasion, and elongation at break have been studied. Attempts have been made to compare fabric properties of partial esters of like DS with method of esterification and effects of a chemically attached alkyl group with the effect of the same group deposited or adsorbed on the surface of the cotton fabric. Consideration has been given to the size of the alkyl group, its orientation on the surface of the fiber, and its hydrophobicity.

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THE EFFECT OF LAUNDERING VARIABLES ON THE FLAME RETARDANCY OF COTTON FABRICS. R. M. Perkins, G. L. Drake, Jr. and W. A. Reeves, Southern Regional Research Lab., New Orleans, La. 70119.

Some durable flame retardant finishes for cotton fabrics can become ineffective if improper laundering procedures are used. For example, one flame resistant fabric will fail to pass the standard vertical flame test after 5, 10 or 15 soap launderings (yet show no reduction in phosphorus content) while another fabric treated with a different formulation will remain flame resistant. Synthetic detergents, rather than soap chips, and soft water have been recommended for some THPC-based flame retardant fabrics to prevent a lime-soap deposit which impairs performance. The effect of these laundering variables has been studied in relation to a variety of different types of durable flame resistant fabrics.

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THE USE OF SURFACE ACTIVE AGENTS IN TANNING OF LEATHER. E. M. Filachione, Eastern Regional Research Lab., ARS, USDA, Philadelphia, Pa. 19118.

Leather is a very unusual fabric. It is a stabilized form of the fibrous protein collagen, which is the main constituent of animal hides and skins. This natural raw material possesses a unique structure, a network of intricately interwoven fibers. There are about 25 operations in the process for converting hides and skins into leather. Surface active agents are used in various steps in this conversion. Surface active agents are of considerable importance as emulsifiers in the tannery process known as fatliquoring. Fatliquoring is the process for lubricating the tanned fiber to which it imparts strength, suppleness, softness and feel. Fatliquoring involves the controlled breaking of an oil-in-water emulsion to obtain proper distribution of the lubricant in the leather. Sulfated oils are by far the most commonly used agents. They may be used alone or blended with other anionic, cationic or nonionic or both surfactants to give better control over the deposition of the lubricant. Surface active agents are also used, though to a lesser extent, in the following processes: soaking, solvent degreasing, tanning, dyeing, impregnation and finishing. Some specific surface active agents such as chrome or other complexes of fatty acids or perfluoro fatty acids, and long chain alkenyl succinic acids, have also been employed to impart water repellent properties to leather. In recent years research has indicated some promising new applications for surface active agents. These include the use of long chain quaternary ammonium salts as short term preservatives of fresh hides and of an amphoteric long chain fatty amino acid as a lubricant resistant to drycleaning.

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THE INFLUENCE OF SURFACE ACTIVE AGENTS ON THE BEHAVIOR OF WOOL FIBERS UNDERGOING FELTING. Dirk Stigter, Western Regional Research Lab., Albany, Calif. 94710.

Surface active agents in aqueous solution can exert characteristic influence on the response of wool fibers to mechanical agitation. Three related interactions are involved: between detergent and water, between wool and water, and between wool and detergent. In all cases the main interactions are of a hydrophobic nature or of electrostatic origin or both. The theory of micelle formation of ionic detergents in aqueous salt solutions is reviewed briefly and shown to yield estimates of the electrostatic and of the nonelectrostatic components of the intermolecular interaction energies. The felting of wool in water is regarded as a flocculation of wool fibers promoted by mechanical agitation. This approach explains the felting behavior of natural wool and of chemically modified wool in terms of the chemistry of the fiber surface. Finally, the adsorption energy of detergents at the wool fiber/water interface is discussed. In the felting process the molecular adsorption energy of the detergent must compete with the mechanical forces between agitated fibers which forces tend to remove the adsorbed detergent from areas of interfiber contact. Such competition explains some observed effects of detergents on the felting shrinkage of wool fabrics.

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THE SELECTION OF EMULSIFIERS FOR USE IN FORMULATION OF "SPIN FINISHES" FOR MAN-MADE FIBERS. J. P. Redston, W. F. Bernholz, R. C. Nahta, Drew Chemical Corporation, Boonton, N.J.

The demands made upon chemicals used as emulsifiers for natural fiber finishes have been minimal, apart from their prime function of forming a satisfactory emulsion with the lubricant, water repellent, or softener. Natural fibers have built-in spinning lubricants or protective sizing. The introduction of polyamides, acrylics, polyesters and polyolefins has required increasingly detailed information from the surfactant manufacturer about his products. A "spin finish" is applied to synthetic linear polymers soon after extrusion. Manipulation of such fiber assemblies into yarn and fabric is possible only after the "spin finish" is present on fiber surface. Chiefly the "spin finish" comprises a lubricant and an antistatic agent which are chosen for their specific properties. Their performance in the mixture must often be enhanced and expanded by the careful choice of emulsifiers whose unique physical properties must be recognized and defined. In practice, straight chain fatty acid triglycerides can offer effective fiber to metal lubricity while incorporating thermal stability greater than that of mineral oil. Such performance is often exceeded by complex polyol esters with tertiary carbon configuration in the polyol. Hence, apart from the ability to properly emulsify the lubricant and blend with the antistatic agent, supplemental physical properties of the emulsifier are often crucial to the success of the blend. These new demands are being met to a very large degree by products based on vegetable fats and oils. Examples will be given of the effect of the emulsifier in contributing to the physical properties of the composite finish upon the fiber. A new, inexpensive, but useful class of emulsifiers based on fatty raw materials will be introduced for potential "spin finish" applications. They incorporate triglyceride lubricating properties and useful surfactant-auxiliary antistat properties not found in fiber finish systems currently available to the fiber processor.

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STUDIES ON THE ENZYMATIC SYNTHESIS OF CHOLESTEROL. T. J. Scallen, M. W. Schuster and A. K. Dhar, The University of New Mexico School of Medicine, Albuquerque, N.M. 87106.

We have demonstrated the enzymatic conversion of squalene to cholesterol by an acetone powder of rat liver microsomes supplemented with the 105,000 × *g* supernatant of rat liver. This report will review several studies which have utilized the acetone powder preparation; the results obtained shed new light on the biological mechanisms involved in the enzymatic synthesis of cholesterol. Specifically, a pyridine nucleotide requirement has been demonstrated for the conversion of Δ^{7}-cholestenol to $\Delta^{5,7}$-cholestadienol. In addition, using a washed acetone powder preparation, a requirement has been demonstrated for the 105,000 × *g* supernatant of rat liver in four separate reactions between squalene and cholesterol. Many experiments suggest the hypothesis that the 105,000 × *g* supernatant of rat liver contains a noncatalytic "carrier" protein which originates from the endoplasmic reticulum, binds the substrate, and makes the substrate reactive to the sterol synthesizing enzymes present in the acetone powder. Evidence will be presented which suggests that this may be an important general mechanism in the biosynthesis of cholesterol.

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ISOLATION OF THE PARTICLE-BOUND ENZYMES OF STEROL BIOSYNTHESIS. J. L. Gaylor, N. J. Moir, R. W. Topham and W. L. Miller, Graduate School of Nutrition, Cornell University, Ithaca, N.Y. 14850.

Investigation of the enzymic reactions of sterol biosynthesis has been approached by: (a) synthesizing appropriate sterols and investigating metabolism of the substrates in crude enzyme systems (organic approach); and (b) developing assays for component enzymes and isolating individual enzymes from the crude systems (enzymic approach). In this laboratory we are using the enzymic approach of isolation and purification of particulate enzymes of sterol biosynthesis. To date, nine enzymes have been isolated and carried through various stages of purification. Methods of isolation include the release of soluble enzymes from particles that have been extracted with solvents or desiccated or both, suspended in media containing ionic or nonionic detergents, digested partially with phospholipase or proteinases, or shocked with changes in ionic strength. Further, some enzymes may be extracted from untreated microsomes with the appropriate buffer. Examples of these methods and some difficulties and pitfalls will be given. The initial goal of these studies is to assemble the biosynthetic reactions in a logical sequence. Arguments based on substrate specificities of the purified enzymes will be presented. A second goal is to examine each enzymic reaction mechanistically with the purified enzyme. One intensive study will be described. The third goal, and to date it is in the future, is to come to grips with the elusive question of whether or not subcellular organization of these enzymes in membranous systems imparts efficiency, specificity, or control that otherwise would be lost. Experimental approaches to the third goal will be presented briefly for the purpose of discussion.

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REGULATION OF THE SEQUENCING IN STEROL BIOSYNTHESIS. W. R. Nes, Drexel Institute of Technology, Philadelphia, Pa.

In an attempt to shed light on the regulation of the sequencing in the sterol pathway and on the latter's relationship to species differences and the evolutionary process, this Laboratory has studied biosynthetic events in various organisms. After epoxysqualene there appear to be several major points of bifurcation or of other control. Among these are (a) the formation of lanosterol or cycloartenol, (b) enzymes which are or are not compatible with further metabolism of the latter two compounds, and the presence or absence of an isomerase for opening of the 3-membered ring, (c) the reduction or alkylation of the Δ^{24}-bond, (d) formation of a *cis*- or *trans*-ethylidene group, (e) introduction (or not) of a Δ^{22}-bond, and (f) reduction or not of the Δ^{22}- or $\Delta^{24(28)}$-bonds or both. In addition, (g) steps can be added or deleted in a given organism. We have obtained the following information on these points. (a) Cycloartenol and lanosterol are metabolically equivalent in vivo in corn seedlings yielding the same Δ^{5}-sterols in essentially the same yields. This indicates along with evidence from other laboratories that an isomerase exists at some point in the pathway and that the choice of one of the C_{30}-compounds as a precursor to sterols in higher plants resides exclusively at the cyclization of epoxysqualene. (b) In pea homogenates cycloartenol, lanosterol and desmosterol were all converted in about the same yield to their respective 24-methylene derivatives indicating that the alkylase is not absolutely specific relative to the point in the pathway at which it operates. Perhaps more importantly it indicates that the choice of cholesterol vs. 24-alkylcholesterol as a final product is not made through formation of lanosterol vs. cycloartenol but that some other regulatory factor is operating for the choice of reduction vs. alkylation. (c) Mevalonate leads in pine seeds and corn seedlings to accumulation of a diene which is an intermediate to 24-ethylcholesterol. It was identified in the pine as 28-isofucosterol. In protozoa 28-isofucosterol but not fucosterol led to the $\Delta^{5,7,22,24(28)}$-tetraene. This stereospecificity suggests that the formation of the saturated side chain occurs via the trans-trans-$\Delta^{22,24(28)}$-structure and that the cis-$\Delta^{24(28)}$-bond is incompatible with formation of the saturated side chain due to steric hindrance of Δ^{22}-introduction. (d) Mammals as exemplified by rats have been found incapable of de novo phytosterol biosynthesis, to lack a $\Delta^{24(28)}$-reductase, and to possess an additional enzymatic system capable of utilizing C_{28} of preformed 24-methylenecholesterol for cholesterol formation in 19% yield in vivo. (e) Sterols and squalene were found in blue-green algae indicating that the entire biosynthetic pathway evolved prior to the evolution of eucaryots.

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DEMETHYLATION OF LANOSTEROL: RECENT FINDINGS CONCERNING THE REMOVAL OF 4,4'-METHYL SUBSTITUENTS. R. B. CLAYTON, Stanford University School of Medicine, Stanford, Calif. 94305.

Earlier studies involving the in vitro hepatic metabolism of 4-substituted cholestanol derivatives, will be briefly summarized. They have shown that in these analogs of the natural series of intermediates in the conversion of lanosterol to cholesterol, the 4α-methyl group of a 4,4'-dimethyl sterol is removed first. It has also been shown that enzymic epimerization of the 4β-methyl into the 4α-configuration occurs when the substrate is in the 3-keto-form. These results suggest that in the demethylation of 4,4'-dimethyl-Δ^8-cholestenol the 4α-methyl group is lost first and that the 4α-methyl of the C_{28} sterol intermediates in its conversion to cholesterol originally occupied the 4β-position. The assumption that the biosynthesis of lanosterol from mevalonate-5-^{3}H leads to labeling of the 4α-methyl group has recently been confirmed in our laboratory. Mevalonate-2-^{14}C-5-^{3}H was used as the substrate for aerobic incubations of rat liver homogenates from which squalene, lanosterol, 4α-methyl-Δ^7-cholestenol and cholesterol were isolated by TLC and GLC. The $^3H/^{14}C$ ratios were: squalene 10.67, lanosterol 10.67, 4α-methyl-Δ^7-cholestenol 12.78, cholesterol 11.63. Carrier isolation and purification of the 4α-methyl sterol and its conversion to the corresponding 3β,7α,8α-triol confirmed the identity of the product and the constancy of its isotope ratio. The change in isotope ratio in the conversion of lanosterol to 4α-methyl-Δ^7-cholestenol is consistent with loss of 4α-methyl prior to 4β-methyl, but not with the reverse order of demethylation.

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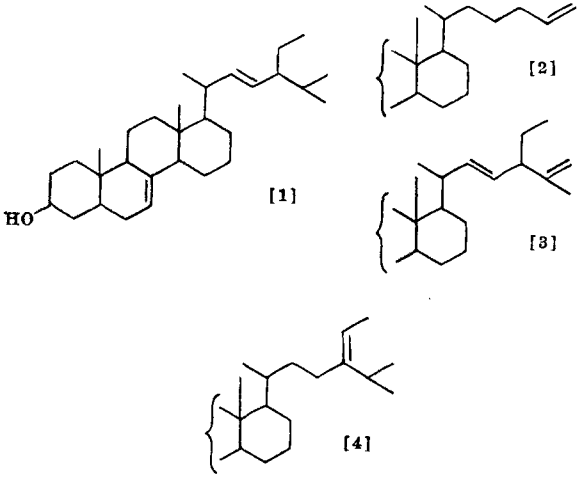
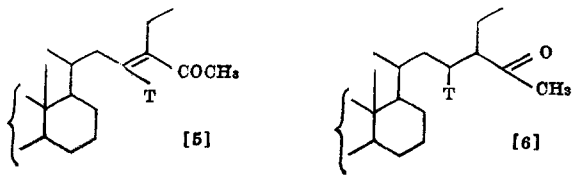
BIOSYNTHETIC FORMATION SEQUENCE OF PUMPKIN STEROLS. WOLFGANG SUCROW and BERND RADÜCHEL, Org. Chem. Inst. Technische Universität Berlin, Germany.

Pumpkin (*Cucurbita pepo* L.) contains α-spinasterol [1], 5α-stigmasta-7,25-dien-3β-ol [2], 5α-stigmasta-7,22,25-trien-3β-ol [3], and 5α-stigmasta-7,24(28)Z-dien-3β-ol [4]. Evidence is



now presented that [4] is an intermediate in the biosynthesis of [3] and [2], but not directly of [1]. Tritium labeled [4], activity 8.5×10^7 ipm/mg (prepared by T_2O equilibration of 3β-hydroxy-5α-stigmast-7-en-24-one and subsequent Wittig reaction with triphenyl ethylidene phosphorane) was fed to pumpkin seedlings. After five days the sterols [1] to [3] were isolated and indicated incorporation rates of 0.12% for [3], 0.08% for [2], but only 10^{-3}% for [1]. The activities were measured at the ketones [5] and [6], obtained by OsO_4/HJO_4 degradation from [3] and [2] respectively and



crystallized to constant activity, and at the ozone cleavage product (methyl-ethyl-butyraldehyde as DNP) of [1], whereas the sterol aldehyde was free of activity. A possible biosynthetic scheme is discussed which involves either formation of [3] from [4] and of [2] from [3] or formation of both [2] and [3] from [4] with a common intermediate. In accordance with other authors, [1] could be formed via the sterol with saturated side chain (5α-stigmast-7-en-3β-ol) which, however, was not yet detected in pumpkin.

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CONJUGATION OF POLYUNSATURATED FATS: II. AS METHYL LINOLEATE WITH TRIS(TRIPHENYLPHOSPHINE)RHODIUM CHLORIDE. W. J. DEJARLAIS and L. E. GAST, Northern Regional Research Laboratory, Peoria, Ill. 61604.

Others have observed tri(triphenylphosphine)rhodium chloride (I) catalyzes the conjugation of certain vinyl ether-containing cyclic dienes by refluxing them in chloroform or benzene solution. Since this treatment is particularly mild compared to conjugation of methyl linoleate with such catalysts as *cis*-bis(triphenylphosphine)platinum chloride and stannous chloride, we investigated its effect on the conjugation of methyl linoleate. Oxygen must be rigorously excluded from the reaction mixtures to prevent deactivation of the catalyst. The reaction, conducted under nitrogen is much more rapid in methanol than in chloroform. Under hydrogen, the initial rate of conjugation increases in chloroform over than in chloroform under nitrogen but is still less than in methanol under nitrogen. Hydrogenation to monoene occurs fairly rapidly in methanol under hydrogen at 65 C but does not occur under nitrogen with similar conditions. Under long reaction times (24 hr) and hydrogen at 65 C about equal amounts of stearate and monoene are formed with about 5% nonconjugated diene remaining. Under similar conditions in chloroform a trace of stearate is formed, along with 18% monoene, 50% conjugated diene and 32% nonconjugated diene. Bis(triphenylphosphine)rhodium chloride dihydride has been postulated as both a hydrogenation and isomerization agent. The activation by hydrogen of I as conjugation and hydrogenation agent in chloroform agrees with this postulate. Our results in methanol do not support this supposition since disappearance of nonconjugated diene is about as rapid under nitrogen as under hydrogen. The dihydride complex must be able to form as readily under nitrogen as under hydrogen and yet under nitrogen no reduction occurs, only isomerization.

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A SIMPLIFIED ASSAY FOR CHAIN-ELONGATION AND ITS APPLICATION TO THE ELONGATION OF 14:0 THROUGH 19:0. J. R. PAULSRUD, GUSTAV GRAFF, SHEILA S. STEWART and R. T. HOLMAN, The Hormel Institute, Austin, Minn. 55912.

The classic method for microsomal chain-elongation has involved the exclusion of O_2 to prevent simultaneous acyl desaturation. Because CN^- inhibits the desaturase but not chain-elongation, an aerobic system has been developed for pseudo-first order rate studies of the chain-elongation of the saturate series of fatty acids 14:0 to 19:0. In addition, a rapid assay system has been developed to replace radio-GLC. The separation of the substrate and its elongated product is performed by reversed phase thin layer chromatography. Comparative rates of chain-elongation of the saturate series in normal and EFA-deficient rat liver microsomes will be presented.

84

AUTOXIDATION OF LIPIDS IN ATMOSPHERES CONTAINING NITROGEN DIOXIDE AND OZONE. J. N. ROEHM and D. B. MENZEL, Battelle-Northwest Laboratories, Richland, Wash. 99352.

Purified methyl esters of unsaturated fatty acids were exposed to atmospheres containing 0.1 to 5.0 ppm of either nitrogen dioxide or ozone. The course of oxidation was determined using the 2-thiobarbituric acid (TBA), ultraviolet spectrophotometric (absorption at 235 nm), and gravimetric analyses. Thin films of methyl linolenate, oxidized in atmospheres containing NO_2, produced TBA values in excess of 500 ppm after 2 hr exposure to 1 ppm NO_2. The air-exposed control, on the other hand, required 33 hr to reach a similar TBA value. The addition of antioxidants decreased the initial rate of oxidation but at these extremely high oxidation rates did not result in a lengthening of the induction period. Ozone was also found to rapidly catalyze autoxidation but to a slightly lesser extent than that observed with NO_2. The ability of these two air pollutants to rapidly catalyze lipid autoxidation, possibly in the lung lining, may be related to their reported influence on obstructive respiratory diseases.

85

THERMAL DECOMPOSITION OF TRISTEARIN. G. P. SHULMAN and E. L. EVANS, Jet Propulsion Laboratory, Pasadena, Calif. 91103.

Effluent gas analysis of tristearin showed that pyrolysis occurred in the range of 400–475 C, but that the temperature range was markedly lowered by blending with certain minerals. From this evidence it was inferred that catalytic decomposition had occurred. Confirmation was obtained by comparing gas chromatograph-mass spectrometer analyses of a tristearin 500 C pyrolysate with those of tristearin mixtures. For the pure material, formation of straight chain alkanes and alkenes, with few rearrangement products, was observed. The oxygen appears mainly as carbon dioxide. Evidence for selective chain cleavage reactions in the vicinity of the carbonyl group followed by decomposition of the resulting olefins was obtained. For the iron-rich mixture, extensive cyclization and dehydrogenation was noted, particularly for the C_5 to C_8 chain lengths, and appreciable isomerization of the long chain alkanes or alkenes also occurred.

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MASS SPECTRAL STUDIES OF TRIGLYCERIDES. A. J. AASEN, W. M. LAUER and R. T. HOLMAN, The Hormel Institute, Austin, Minn. 55912.

The mass spectra of triglycerides will be discussed. Syntheses of specifically labeled deuterium compounds have made it possible to postulate modes of fragmentation.

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SOME THERMAL PROPERTIES OF METHYL MALVALATE, METHYL STERCULATE, AND THEIR DIHYDRO DERIVATIVES. J. L. WHITE, SR., ZIGRIDA ZARINS and R. O. FEUGE, Southern Regional Research Lab., New Orleans, La. 70119.

The methyl esters of the cyclopropene acids, malvalic and sterculic, and of their dihydro derivatives were highly purified and then examined for melting point, polymorphic behavior, heats of fusion, and dilatometric behavior. The capillary melting points of methyl malvalate and methyl sterculate were −22.5 and −14.3 C, respectively. Both dihydro compounds melted at about −3.5 C. Examination of methyl malvalate and methyl sterculate in capillary melting point tubes and in a differential scanning calorimeter revealed that quickly chilled forms of each underwent a single, rapid, monotropic transformation. The transformation of methyl malvalate occurred at about −48 C with a heat of transformation of −1.49 K calories per mole. Methyl dihydromalvalate and methyl dihydrosterculate exhibited no detectable polymorphism. Heats of fusion were determined for all four compounds. Expansivities in the solid and liquid states and melting dilations also were determined. The melting dilation for methyl malvalate, 0.0900 ml/g, was significantly below that of other methyl esters of fatty acids reported heretofore.

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THE STRUCTURAL CONSTITUENTS OF CARNAUBA WAX. L. E. VANDENBURG and E. A. WILDER, S. C. Johnson & Son, Inc., Racine, Wis. 53403.

Type 1 yellow carnauba wax has been separated into its

structural constituents. Analysis of these constituents by a variety of conventional techniques has shown the composition to be: hydrocarbon 0.3–1%, aliphatic esters 38–40%, monohydric alcohols 10–12%, omega hydroxyaliphatic esters 12–14%, para methoxycinnamic aliphatic diesters 5–7%, para hydroxycinnamic aliphatic diesters 22–25%, a free triterpene diol 0.4%, and free acids and other unknown minor constituents 5–7%. Type 4 carnauba wax, the common wax of commerce, was found to be essentially the same as Type 1 wax except the cinnamic esters were highly polymerized and none of the free triterpene diol could be isolated. Chemical constants and infrared spectra of the identified specimens are included.

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ODOR AND FLAVOR RESPONSES TO ADDITIVES IN EDIBLE OILS. C. D. Evans, Helen A. Moser and G. R. List, Northern Regional Research Lab., Peoria, Ill. 61604.

The odor threshold was determined for a series of unsaturated ketones, secondary alcohols, and hydrocarbons, as well as a series of substituted furans in bland edible oil. Odor thresholds were taken at the point where 50% of a 15–18 member taste panel could detect an odor difference from the control oil. Reportedly, these additives or some of their analogs are oxidative products of fats, but the concentrations investigated were far below any level associated with an identifying odor or taste of the additive per se. Odor, rather than flavor, was selected as the starting basis because of greater activity and ease of handling a large number of samples with less taster fatigue. Oil samples containing additive concentrations near the odor threshold levels were evaluated by flavor score and flavor descriptions. Taste panel members were experienced oil tasters and were allowed free choice in selecting terms to describe the flavor quality of the oil samples. The propyl and butyl isologs in the series of ketones and alcohols had markedly low thresholds, whereas the difference was small between isologs in the 2-substituted furans. Vinyl propyl ketone, vinyl propyl carbinol, and 2-propyl furan had odor thresholds of 0.005, 0.5 and 5.7 ppm, respectively. The odor thresholds of the unsaturated hydrocarbons are markedly lower than the saturated homologs. The odor of nonane can be detected at 650 ppm. However, at 1000 ppm it cannot be tasted and oils containing it were scored equal to the control oil. 1-Nonene, 1-nonyne, and other C_9 unsaturated hydrocarbons, including a number of dienes, have odor thresholds of about 10 ppm. The hydrocarbons, 1-hexyne, 1-nonyne, and 1-decyne had odor thresholds of 0.2, 2.2 and 4.3 ppm, respectively. Flavor descriptions, odor thresholds, methodology, panel and taster acuity and reproducibility will be presented and discussed for this series of compounds concerned with oxidized fat decomposition products.

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RANCIDITY OF SOYBEAN OIL BY GAS CHROMATOGRAPHY AND ITS RELATIONSHIP WITH PEROXIDE VALUE AND FLAVOR EVALUATION. P. K. Jarvi, G. D. Lee, D. R. Erickson and E. A. Butkus, Swift & Co. R&D Center, Oak Brook, Ill. 60521.

The rancidity of soybean oil has been studied using the techniques of gas chromatography, peroxide value, and sensory evaluation. The gas chromatographic procedure has been adapted for the purpose and a new GLC column packing was developed and applied. The pattern of gas chromatographic peaks have been treated as one whole group, and by means of an internal standard (*n*-octanol) an oxidation value has been computed. The oxidation values have been shown to be in good correlation with the peroxide values. The flavors of the soybean oil along with a blend composed of soybean oil and hydrogenated soybean oil (kept at 140 F for varying intervals of time) have been evaluated by a panel using the ranking method. The results are presented in a new graphical form. A relationship between the oxidation values and the sensory panel's flavor evaluation of the oils studied has been shown to exist. The possible applications and merits of these studies are discussed.

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A NEW METHOD FOR EDIBLE OIL REFINING BY ADSORBING FREE FATTY ACIDS ON A BED OF AN ADSORBENT MATERIAL. R. L. Husch, Interstate Foods Corporation, Chicago, Ill. 60609.

Laboratory work has been carried out during the last year on the adsorption of free fatty acids, on a column of adsorbant material with excellent results. A pilot plant composed of a column of adsorbant material capable of producing 500 lb/hr of refined oil is in operation at Interstate Foods Corporations' Plant, Chicago, Illinois to determine the required operational conditions and equipment for full scale production. A review of this pilot plant, its composition, method of operation, and advantages over standard methods of refining is presented in this paper. Laboratory and pilot plant data are given and some of the available theory of adsorption and its relation to the above process is discussed.

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FUNDAMENTAL ASPECTS OF BLEACHING FATTY OILS. A. D. Rich, Bennett-Clark Co., Inc., Nacogdoches, Tex. 75961.

Various technical aspects involved when fatty oils are bleached, in the form of questions and answers will be discussed. Included are the following: (a) Nature and function of bleaching clay: types of clay, mechanism of bleaching reaction, adsorptive capacity of clays, oil impurities removed, red vs. green color removal, effect of bleaching upon organic peroxides, flavor and odor. (b) Optimum bleaching conditions: temperature, time, plant vs. laboratory bleaching, vacuum vs. atmospheric bleaching. (c) Effect of clay characteristics upon aspects other than decolorizing: effect upon FFA rise, oil retention of cake, cloth deterioration and filtration rate. (d) Effect of oil characteristics upon decolorizing: effect of moisture, color, organic impurities other than color pigments, oxidative state and iodine value.

93

FILTERS AND FILTRATION SYSTEMS FOR THE VEGETABLE OIL INDUSTRY. Frank Passalaqua, Industrial Filter & Pump Mfg. Co., Cicero, Ill. 60650.

This paper will discuss new innovations of equipment and its use in the various vegetable oil processes. The ultimate goals are reduction in plant space requirements, lower operating costs, upgrading of product being processed, reduced labor expense, increase of in-plant cleanliness. The processes to be investigated are: (a) Deodorization—the adaptation and use of a vertical tank tubular filter featuring paper dressed elements. (b) Clay bleaching—the use of horizontal tank vertical leaf filters using a wire cloth filter medium. A method of operation which does not require a precoat. (c) Nickel catalyst—the utilization of a horizontal tank, vertical leaf filter used in conventional hydrogenation systems using a method of operation which eliminates the need for precoating and prevents the blinding of wire cloth filter media. A new approach to the filtration of nickel catalyst at elevated temperatures which improves total throughput and reduces the amount of time required to complete the hydrogenation process. This increases the overall production of existing converters. The use of conventional post bleach tanks can, in some cases, be eliminated. This new system covers the handling of the oil from the converters straight through to storage and is adaptable to a package purchase concept. (d) Tank loading—a vertical tank tubular filter found to be extremely economical to operate at high flow rates, no unfiltered heel, one man operation. These units are becoming very popular for use as tank loading filters and are excellent for general filtration throughout the plant.

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STEAM JET EJECTORS. R. E. Richenberg, Graham Manufacturing Co., Inc., Batavia, N.Y.

The history, development and operating principle of the steam jet ejectors will be covered with special emphasis on recent advances in design. Selecting the type of equipment applicable to process requirement will be discussed such as number of stages being used and whether barometric or surface type of condensers could be used. Since the consumption of steam versus water can be varied the cost and economics of each system will be studied. Start-up and shut-down procedures will be mentioned with thorough study given to trouble shooting as applicable in plant operations.

95

CHOLESTEROL TURNOVER IN INSECTS. H. E. Vroman and J. N. Kaplanis, Claflin University, Orangeburg, S.C. 29115.

In an investigation of the turnover of cholesterol in insects, we fed 4-^{14}C-cholesterol to a group of newly emerged, adult male American cockroaches, *Periplaneta americana,* for 60 days. Periodically some roaches were removed from the group and the specific radioactivity of the total cholesterol was determined. We found that the turnover was linear and relatively slow, for in the 60 days only 40% of the cholesterol had been replaced by the labeled dietary cholesterol and equilibrium between the dietary cholesterol and the cholesterol of the tissue had not been reached. We then continued this investigation in two directions: first in an insect with a short life span, the male housefly, *Musca domestica,* and second, in a long-term study of *P. americana.* We found in the investigation of *M. domestica* that the turnover was linear over the life span of this insect and that there was an overall loss of cholesterol from the tissues. The concentration of esterified cholesterol and free cholesterol declined in different manners. In the 225 day investigation of turnover in *P. americana* the concentration of cholesterol in the carcass rose initially, apparently because of a rise in the concentration of free cholesterol, and then declined gradually. The concentration of esterified cholesterol, on the other hand, declined throughout the experimental period. Our results show that the pattern of turnover of cholesterol in the insect, if not linear, approaches linearity. The absence of the complication of cholesterol biosynthesis in insects may be an important factor in determining this pattern. In addition we found that at least 95% of the cholesterol in the carcass of *P. americana* is replaceable by dietary cholesterol. Our results suggest that 95% may be the maximum replaceable figure. In both *P. americana* and *M. domestica* we found a decline in the concentration of cholesterol, and the esterified cholesterol concentration declined in a manner different from that of the free cholesterol.

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UTILIZATION AND METABOLISM OF STEROLS IN THE SILKWORM, *BOMBYX MORI.* Toshio Ito, Yasuhiro Horie and Shoichi Nakasone, Sericultural Experiment Station, Wada, Suginami-ku, Tokyo, Japan.

The silkworm is unable to synthesize sterols and requires them from dietary sources. Utilization of β-sitosterol by the silkworm is highly accelerated in the presence of dietary fatty acids, and the sparing of sterol in the presence of fatty acid was considered to be a metabolic function. However, the physiological mechanism of the sparing effect has remained unsolved. The sparing effect was confirmed with cholesterol and stigmasterol. The optimal amounts of sterol required in the presence of the refined soybean oil were far smaller than those in the absence of the oil. Cholesterol and β-sitosterol were equally effective at a low dietary level (0.2%) in the presence of oil, whereas are 0.8% level of stigmasterol was required. Subsequently, 4-^{14}C-cholesterol was administered orally to larvae. Feces were collected, and the radioactivity was determined. The rates of absorption of labelled cholesterol varied according to the cholesterol levels of the basal diets, but significantly high absorption rates were obtained in the absence of oil. In the absence of oil, more radioactivity was accumulated in the intestine than in the presence of oil. However, in the presence of oil the radioactivity in the blood appeared more quickly than in the absence of oil. Furthermore, the percentages of esterified sterol were high in the presence of oil, both in the intestine and blood. Sterol composition of the larva varied according to the different dietary sterols. The conversion of sterol with 29 C-atoms into C_{27}-sterol was also recognized.

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UTILIZATION AND METABOLISM OF STEROLS IN *DROSOPHILA.* H. W. Kircher and Kenneth Goodnight, Department of Agricultural Biochemistry, University of Arizona, Tucson, Ariz. 85721.

Insects are useful animals for the study of sterol metabolism. Unlike vertebrates and plants, they cannot synthesize sterols from basic foodstuffs, but must ingest them in their diet. All of the insects reported to date, except one, can utilize cholesterol as the dietary sterol. Indeed, even in the absence of dietary cholesterol, this compound is often the major tissue sterol. *Drosophila melanogaster* and *D. pachea* eggs were collected and surface sterilized with 0.5% peracetic acid. The eggs were placed on sterile media and the adults which formed were transferred aseptically to fresh media. This,

together with continuous microbiological assay, maintained a continuous axenic culture of the two species throughout the study. A diet of negligible sterol content (0.002% of dry ingredients per liter of medium) was developed from solvent extracted yeast, soy protein hydrolyzate, fructose and agar together with carboxymethyl cellulose and vitamins. With this sterol deficient basal diet it was conclusively shown that *D. pachea* could not utilize cholesterol for growth and development. *D. melanogaster* grew well on the medium with added cholesterol or lathosterol (7-cholesten-3β-ol) but did not develop to maturity with lophenol (4α-methyl-7-cholesten-3β-ol) as the dietary sterol. This is the first demonstration of the inability of an insect to demethylate a 4α-methyl sterol. Axenic *D. pachea* were collected that had developed to maturity on the medium plus 0.25% lathosterol. The sterol fraction from these flies contained only lathosterol and 7-dehydro-cholesterol. It has been shown with several insects that the $\Delta^5 \rightarrow \Delta^{5,7}$ and $\Delta^0 \rightarrow \Delta^7$ sterol desaturation pathways are operative and it has been suggested that the pathway from $\Delta^5 \rightarrow \Delta^{5,7}$ sterols involves the following steps: $\Delta^5 \rightarrow \Delta^0 \rightarrow \Delta^7 \rightarrow \Delta^{5,7}$. We have shown the last step in this sequence to be present in *D. pachea*.

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THE INHIBITIVE EFFECTS OF AZASTEROLS ON STEROL METABOLISM AND GROWTH AND DEVELOPMENT IN INSECTS WITH SPECIAL REFERENCE TO THE TOBACCO HORNWORM. J. A. SVOBODA and W. E. ROBBINS, Insect Physiology Lab., USDA, Beltsville, Md. 20705.

We have previously shown that the two diazasterols, 22,25- and 20,25-diazacholesterol inhibit sterol metabolism and normal development in the tobacco hornworm, *Manduca sexta* (Johannson). Subsequent studies with a number of azasterol analogs and derivatives have provided information on the relationship of structure to activity of the azasterols with respect to both inhibition of the Δ^{24}-sterol reductase enzyme system(s) and the disruption of larval development of the hornworm. Several monoazasterols that are considerably more potent than either of the above two diazasterols prevent normal pupation in the hornworm at dietary concentrations in the ppm range. The primary effects of these azasterols on larval development include the inhibition of the molt from the third to fourth larval instar, a prolongation of the molting cycle and the formation of prepupae an instar early (4th instar) that are unable to pupate. In addition, comparative studies of effects of feeding a diazacholesterol in combination with β-sitosterol to several species of insects that are capable of dealkylating phytosterols revealed considerable differences in the effects of the diazasterol both on sterol metabolism and growth and development.

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STEROIDS OF MOLTING HORMONE TYPE FROM ANIMALS AND PLANTS: CHEMISTRY AND BIOCHEMISTRY. HANS HOFFMEISTER, Bundesgesundheitsamt, 1000 Berlin 33, Postfach, West Germany.

The development of insects and other arthropods is essentially regulated by two steroid hormones, ecdysteron and ecdyson. The hormones have been isolated from insects and crustaceans and cleared up in their structure. More than 20 steroids, posessing the same or similar structures as ecdysteron have recently been found in the plant kingdom. All steroids with molting hormone activity possess a 6-keto-7-en structure and a side chain like or similar to that of cholesterol. The different hormones bear between four and seven hydroxy groups. Biosynthesis of these steroids in insects and plants derives from cholesterol. Leaves and roots of many plant families contain up to a 1000-fold higher amounts of ecdysterone compared with the hormone capacity in arthropods. But the high content is no protection for these plants against insects. We found a very powerful enzymatic system in calliphora, which regulates the molting hormone activity by making a hormone glycoside. Perhaps it is this mechanism, by which some insects protect themselves against the large quantities of hormones in their food. The molecular mechanism of action of the molting hormones is established better than the mechanism of most of the vertebrate hormones; the earliest known answer to ecdysterone is the formation of some specific puffs in salivary gland giant chromosomes from chironomids. The mechanism of the puffing phenomenon will be discussed.

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LIPIDS OF NORMAL HUMAN ERYTHROCYTES. GEORGE ROUSER and J. D. TURNER, City of Hope Medical Center, Duarte, Calif. 91010.

Quantitative extraction of red cell lipids is accomplished with a series of solvents of increasing polarity. Lipids are then separated from nonlipid contaminants by Sephadex column chromatography which also separates gangliosides from other lipids. Two-dimensional thin layer chromatography which combines high capacity and high resolution makes possible the resolution of even minor lipid components of erythrocytes. The molar amounts of each lipid class are then determined by spectrophotometric procedures (phosphorus assay for phospholipids, sphingosine determination for gangliosides and ceramide polyhexosides, and cholesterol by the zinc chloride-acetyl chloride method). TEAE cellulose (hydroxyl form) column chromatography is used to separate the lipid mixture into groups eluted with: (a) chloroform (cholesterol, triglyceride, cholesterol esters, ceramide); (b) chloroform-methanol 9:1 (choline lipids, phosphatidyl choline, lysophosphatidyl choline, sphingomyelin); (c) chloroform-methanol 2:1 (ceramide polyhexosides including globoside); (d) methanol (no lipid, inorganic substances formed by ion exchange); (e) chloroform-methanol 2:1 + 1% (v/v) glacial acetic acid (phosphatidyl ethanolamine, free fatty acid, and lysophosphatidyl ethanolamine when present); (f) glacial acetic acid (phosphatidyl serine); (g) methanol (wash to remove acetic acid); (h) chloroform-methanol 4:1 made 0.1 *N* in potassium acetate and containing 20 ml of 28% aqueous ammonia per liter (phosphatidyl inositol, phosphatidic acid, numerous uncharacterized acidic phospholipids). Quantitative separation of phosphatidyl serine from other acidic lipids is achieved by passage of fractions 6–8 through a DEAE cellulose column. Ion exchange cellulose column chromatography is advantageous for detection and accurate analysis of minor components and convenient for use when the fatty acid composition of each lipid class is desired. The chromatographic procedures will be described as will the importance of avoidance of artifact production by evaporation to dryness and other precautions.

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THE COMPOSITION OF BLOOD LIPIDS IN NON-HUMAN, MAMMALIAN SPECIES. G. J. NELSON, Lawrence Radiation Laboratory, University of California, Livermore, Calif. 94550.

The erythrocyte and plasma lipids in several nonhuman, mammalian species, including aquatic mammals, have been studied. The analyses were performed using modern procedures such as dextran gel purification of total lipid extract and two-dimensional, thin layer chromatographic separation of the individual lipids coupled with spectrophotometric phosphorus determinations. The erythrocyte lipids are characterized by rather wide variations in proportions of the various phospholipids present, while the plasma phospholipids show only small deviations from a standard pattern for all the species studied. Ruminants, for example, have little or no lecithin in their erythrocytes yet lecithin is the predominant phospholipid of the plasma. Also a new phospholipid, *N*-acyl phosphatidyl serine, not previously reported in any tissue, has been isolated from sheep erythrocytes. This substance has also been detected in cow and goat red cells and in trace amounts in the erythrocytes of other species. It is not present in plasma. Certain differences between the results obtained in this work with those in the literature will be discussed with particular reference to methodology, such as sampling and analytical procedures. In addition to the compositional data, the lipid metabolism of the erythrocyte will be considered with respect to the de novo synthesis of intact phospholipids by mature erythrocytes and the incorporation of inorganic phosphate, as well as exchange of lipids between the cell and the plasma lipoproteins.

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LIPOPROTEINS AND PROTEINS OF ERYTHROCYTES. BERNARD SHORE and VIRGIE SHORE, Lawrence Radiation Laboratory, University of California, Livermore, Calif. 94550.

Several different polypeptide constituents of red cell membranes have been isolated and characterized by physical and chemical means. Some of the peptides, accounting for 25–30% of the total membrane protein, appeared to be only loosely, if at all, associated with lipid in the membrane. The roles of the isolated polypeptides in the structure and function of the red cell membrane are largely unknown. The physical and chemical properties of the polypeptides and possible interactions among the peptides, lipids and carbohydrates will be considered. Evidence for molecular organization and membrane substructure is derived largely from physical and chemical studies on the intact membranes and unfractionated components. These studies also provide evidence for the existence of regions of the membrane in which protein-protein interactions, rather than protein-lipid interactions, predominate.

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LIPIDS OF NORMAL HUMAN PLASMA. J. D. TURNER and GEORGE ROUSER, Department of Medicine, Baylor College of Medicine, Houston, Tex. 77025.

Quantitative extraction of lipids from fasting human plasma is achieved with cold chloroform-methanol 2:1, extraction taking place under nitrogen using solvents containing butylated hydroxytoluene as antioxidant. Lipids are separated from water soluble nonlipid contaminants by Sephadex column chromatography. Two-dimensional thin layer chromatography (TLC) resolves all major plasma phospholipids and reveals the presence of many minor components. Molar concentrations of phospholipids are determined in quadruplicate from TLC plates by phosphorus analysis of spots. Less polar lipids are separated by one-dimensional TLC and determined spectrophotometrically (cholesterol and ester cholesterol by the zinc chloride-acetyl chloride procedure; free fatty acid, cholesterol esters, and triglycerides by the hydroxamic acid procedure after conversion to fatty acid methyl esters; triglycerides by the periodate-chromotropic acid procedure). Ion exchange cellulose column chromatography of plasma lipids with triethylaminoethyl cellulose (hydroxyl form) is useful for concentration of minor components which can then be detected and determined more accurately by two-dimensional TLC followed by spectrophotometric assay. The lipids are eluted with three different solvents to give the following groups: (a) chloroform (less polar or neutral lipids—sterol, sterol ester, triglycerides); chloroform-methanol 9:1 (choline lipids—phosphatidyl and lysophosphatidyl choline, sphingomyelin); chloroform-methanol 4:1 (containing 0.1 *N* ammonium or potassium acetate plus 20 ml/liter of 28% aqueous ammonia (all other phospholipids—phosphatidyl and lysophosphatidyl ethanolamine, phosphatidyl inositol, numerous uncharacterized phospholipids, and free fatty acid).

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SCREENING FOR GLYCERYL ETHERS IN HUMAN POPULATION. J. J. KABARA, STORM SLAVIN and KAREN HEATLEY, Michigan College of Osteopathic Medicine, Pontiac, Mich. 48057.

For a number of years, our laboratory has been interested in the role of lipids in tumor-bearing animals. A survey of the literature, as well as our own work, suggested that certain patterns of lipid metabolism were emerging which hopefully could be associated with the malignancy involved. It should be noted that in the analysis cited by previous workers, as well as our own work, no unusual lipids have been encountered in tumor tissue. Recently however, due to increased sensitivity of lipid methodology, it has been possible to detect, in tumor-bearing animals, lipids which heretofore were not detected. Neutral glycerol ethers in diester form are of particular significance in cancer tissue. The group at Oak Ridge previously reported an unidentified lipid prevailing in tumor tissue, which was subsequently identified as glycerol ether diesters. Further work was done in comparing the appearance of this lipid in mouse, rat and human tumors with normal tissues. In a most recent report, they have given data on the analysis of human tumor tissue in normal organs but not including serum. Evidence to date from our laboratory suggests that, while the tumor-lipid can be found in patients with known diagnosis of cancer, these spots show infrequently in other types of individuals, including normal individuals. The reason for the occurrence of such a spot in these individuals is not known and may be related to genetics, nutrition or drug regimen or both. A better understanding of the role of glycerol ether in human metabolism is necessary before the TLC spot can be used as a successful screening procedure for malignancy in a general population.

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THE SELECTIVE HYDROGENATION OF SOYBEAN OIL METHYL ESTER. J. C. BAILAR, JR., Department of Chemistry, University of Illinois, Urbana, Ill.

Soybean oil is chiefly a mixture of linolenic, linoleic, oleic, stearic and palmitic esters. Since the first and the last two are undesirable components, attempts were made to hydrogenate the oil to the monoene or diene stage, with no reduction to saturation. This has been accomplished by the use of a series of catalysts, the usual one being $[Pt(P\phi_3)_2(SnCl_3)Cl]$. The platinum may be replaced by Pd, the P by As or Sb, the phenyl group by aliphatic groups, and the Sn by Ge or Pb. The catalysts $Mo(CO)_2(PR_3)_2Cl_2$ + $SnCl_2$ and $W(CO)_2(PR_3)_2Cl_2$ + $SnCl_2$ are also effective. The hydrogenated products are monoene esters. All of these catalysts bring about migration of the double bonds, and the double bond in the product may occupy any of a wide variety of positions in the molecule. For the most part, this double bond is *trans*. The choice of solvent is important, chlorinated hydrocarbons (e.g., CH_2Cl_2 and $CHCl_3$) allowing the fastest hydrogenation. Cyclohexadiene, cyclooctatriene, and similar substances are likewise reduced to monoenes, but small, linear molecules such as ethylene and propylene are converted to saturated hydrocarbons. This behavior seems to be characteristic of substances containing terminal double bonds. Nonterminal double bonds in small olefins are also reduced, but only slowly, and small molecules containing conjugated double bonds (e.g., 2,4-hexadiene) are not reduced at all. They form such stable complexes with the catalyst that they render it inactive. A proposed mechanism for the action of these catalysts will be described. Research is now underway to develop heterogeneous catalysts which will selectively hydrogenate soybean oil. These are either polymers of the homogeneous ones or are molecular sieves.

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SOME RECENT DEVELOPMENTS IN THE HOMOGENEOUS CATALYZED HYDROGENATION OF OLEFINS. R. L. AUGUSTINE and JAN VAN PEPPEN, Department of Chemistry, Seton Hall University, South Orange, N.J. 07079.

It has been found that the amount of double bond isomerization accompanying homogeneous olefin hydrogenations over tris-triphenylphosphine rhodium chloride is markedly influenced both by the duration of hydrogen presaturation of the catalyst and the presence of oxygen in the reaction mixture. A mechanistic rational which accounts for these observations will be presented and will be compared to the reaction sequence believed to be occuring over heterogeneous catalysts.

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HYDROGENATION OF NATURAL OILS WITH PLATINUM METAL CATALYSTS. P. N. RYLANDER, Engelhard Industries, Newark, N.J. 07105.

All six elements of the platinum metals group make hydrogenation catalysts and among them catalyze reduction of most functional groups. For hydrogenation of every functional group and for every catalytic side-reaction connected with reduction of the function a sequence of activities for the six elements may be written. The order of the sequence depends largely on the metal with minor variations attributable to the support. In polyfunctional molecules ordering is complicated by the frequently unknown relative tendencies of each function to be adsorbed on the catalyst. For olefins a general activity sequence is Pd>Rh>Pt>>Ir>Ru>Os. The order for double-bond migration, an important factor affecting selectivity is Pd>>Ru>Rh>Pt>>Ir. Selectivity in diolefin hydrogenation decreases in the order Pd>Rh>Ru≅Pt>Ir. Activity for *cis-trans* isomerization decreases in the order, Pd>Rh>Ru≅Ir>Pt. Each of these reactions is influenced by metal and catalyst concentration, agitation, solvent, temperature and pressule. The effects of these variables may be correlated in terms of their influence on hydrogen availability at the catalyst surface. Hardening of fats will be discussed in the above terms.

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THE EFFECT OF REACTION VARIABLES ON ISOMER DISTRIBUTION DURING PARTIAL HYDROGENATION OF TRILINOLEIN WITH COPPER CHROMITE CATALYST. EHUD KIRSCHNER and E. R. LOWREY, The Procter & Gamble Company, Cincinnati, Ohio.

The effect of pressure, temperature and catalyst concentration on the distribution of several diene and monoene isomers in the partial hydrogenation of trilinolein with a copper chromite catalyst has been determined. A description of these effects is given. A previous paper has described differences seen in isomer formation between a nickel and a copper chromite catalyst at one reaction condition. This present paper, a product of the same work, points out a rather surprising lack of effect of process conditions on any single isomer distribution during a hydrogenation run.

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HYDROGENATION OF UNSATURATED FATTY ESTERS WITH COPPER-CHROMITE CATALYST: KINETICS, MECHANISM AND ISOMERIZATION. SAMBASIVARAO KORITALA, Northern Regional Research Laboratory, Peoria, Ill. 61604.

Hydrogenation is important in industrial processing of oils and fats. On the one hand, nickel catalysts, commonly employed to hydrogenate the polyunsaturated fatty acids in vegetable oils, reduce linolenate in soybean oil twice as fast as linoleate (selectivity ratio $K_{Linolenate}/K_{Linoleate} = 2$). On the other hand, copper-chromite catalysts, hydrogenate the esters in soybean oil with a selectivity ratio of 9 to 13. To explain the unusually high selectivity of copper catalysts, model compounds were hydrogenated with copper-chromite at 150 C and atmospheric pressure, and the reaction products analyzed. Products varied considerably depending upon the location of the double bonds in the model compound. Conjugated triene was selectively reduced to conjugated diene. Hydrogenation of linolenate with nickel catalyst gave only traces of conjugated diene, whereas copper-chromite produced mostly conjugated diene. Unlike nickel catalysts, copper-chromite hydrogenates only those compounds whose double bonds are either conjugated or conjugatable (i.e., methylene interrupted). Monoenoates are not reduced by copper-chromite even at 200 C, except when the double bond is in conjugation with the carboxyl group. Binary mixtures of fatty esters were also hydrogenated with copper-chromite. From the composition of the initial and final products, competitive reaction rate ratios were determined. Conjugated double-bond systems were preferentially reduced over methylene-interrupted double bonds. Since conjugation preceeds hydrogenation, several model compounds containing conjugated double-bond systems were hydrogenated with copperchromite and the products analyzed. β-Eleostearate was reduced mostly by 1,6-addition of hydrogen. *Trans,trans* conjugated diene was reduced by 1,4-addition, whereas *cis,trans* conjugated dienes seem to be reduced by 1,2-addition. The double bond distribution in the products formed from linoleate, linolenate and their isomers was consistent with the assumption that the double bonds in polyunsaturated fatty esters first conjugate and then add hydrogen. Extensive isomerization of the conjugated double-bon systems occurred during hydrogenation. *Cis,trans* conjugated dienes were rapidly equilibrated to *trans,trans* conjugated dienes and vice versa. Monoenes were not isomerized under similar conditions of hydrogenation.

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FLAVOR EVALUATION OF COPPER-HYDROGENATED SOYBEAN OILS. C. D. EVANS, HELEN A. MOSER, G. R. LIST and J. C. COWAN, Northern Regional Research Laboratory, Peoria, Ill. 61604.

Flavor and oxidative stability evaluations were made on a series of soybean oils hydrogenated with commercial and laboratory-prepared copper catalysts under a variety of conditions in both laboratory and pilot-plant equipment. Iodine values of the hydrogenated products varied from 104 to 128; linolenic acid content, from 0.0% to 5.5%. The 8 hr AOM peroxide values were below 10 for all hydrogenated oils deodorized with citric acid. The pooled data from all samples showed no relationship between these low oxidative stability values and iodine value or linolenic acid content. Flavor evaluations were made by a 15–18 member taste panel on freshly deodorized oils, on oils stored at 60 C for 4 and 8 days, and on oils submitted to heat tests up to and including frying temperatures. Hydrogenated oil flavor scores for the aged and heated oils were equal to or better than the control, but rarely were they significantly better in all tests. Copper-hydrogenated oils did not show the hydrogenated flavor response typical of nickel-hydrogenated oils. With the marked lowering of linolenic acid in the copper-hydrogenated oils, flavor responses were different and painty-reversion responses were not observed. Quality improvements were noted as the linolenic acid content of the oils approached zero. Residual copper catalyst dissolved in the oil can be effectively removed and copper concentrations reduced below detection levels by bleaching or re-refining after hydrogenation. Oil quality is further improved by the addition of citric acid during deodorization. Flavor responses and oxidative data for typical hydrogenation will be presented and discussed.

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THE UNSAPONIFIABLES OF VERNONIA ANTHELMINTICA SEED OIL. J. A. FIORITI, M. G. KOLOR and R. J. SIMS, General Foods Corp., White Plains, N.Y. 10602.

The unsaponifiable fraction of *Vernonia anthelmintica* seed oil has been studied in detail. Preparative TLC on argentated plates has revealed that the hydrocarbon fraction contains squalene as well as normal odd carbon chain alkanes among which C_{31} predominates. The bulk (>70%) of the unsaponifiable fraction consists of two sterol-like compounds. GLC shows these to be preferentially esterified with linoleic acid. These esters have been isolated from the oil by column chromatography on silica gel and have been separated by preparative TLC. Purification by preparative GLC followed by high resolution mass spectrometry, has shown molecular weights of 410 and 412 with respective empirical formulas of $C_{29}H_{46}O$ and $C_{29}H_{48}O$. The latter corresponds to the already reported Δ-7 avenasterol. The 410 compound is a new sterol which has not been encountered previously. Some of the characteristics of this new stigmastatrienol will be discussed.

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STEROLS IN SAFFLOWER AND OLIVE OILS. D. FIRESTONE, C. W. THORPE, N. L. BROWN and R. P. BARRON, Food and Drug Administration, Department of Health, Education & Welfare, Wash., D.C. 20204.

The unsaponifiable matter in normal (low oleic) safflower oils, high oleic safflower oils and olive oils was fractionated by preparative TLC. The sterols were removed from the TLC plates and examined by GLC. The sterol composition of normal and high oleic safflower oils was identical; the sterols were campesterol (11%), stigmasterol (8%), β-sitosterol (52%) Δ^7-stigmastene-3β-ol (24%) and at least three unidentified components. The presence of Δ^7-stigmastene-3β-ol in safflower oils was confirmed by mass spectrometry. Olive oil sterols were campesterol (3%), stigmasterol (1%), β-sitosterol (86%) and at least three unidentified components. Since no Δ^7-stigmastene-3β-ol was detected in olive oils, GLC analysis can be used to detect adulteration of olive oils with high oleic safflower oil having a similar fatty acid composition.

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CHLOROALKYL SULFATES EXCRETED BY ALGAE. MANUEL POUSADA and T. H. HAINES, City College of The City University of New York, New York, N.Y. 10031.

Chlorosulfolipids have been isolated from the fresh water phytoflagellate, *Ochromonas danica* and from its culture medium. The substances, which are potent detergents, are disulfates of 1,14-docosanediol and 1,15-tetracosanediol with from 0 to 6 chlorines substituting for hydrogens on the chain. After cleaving the sulfate esters the diol mixture was resolved into at least 11 components by a combination of thin layer and gas chromatography. The major diols were hexachlorodocosanediol (34%), chlorodocosanediol (24%) and docosanediol (20%). Other components constituted less than 10% of the mixture. Five of the substances have been completely characterized including the absolute configuration of the chloro and hydroxyl groups. The characterizations were effected by a combination of mass and nuclear magnetic resonance spectrometry, infrared spectrophotometry and optical rotation. The data was confirmed by derivatization and synthesis. It appears that the substances are excreted by many algae and protozoa.

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CYANOLIPIDS OF KOELREUTERIA PANICULATA LAXM. SEED OIL. K. L. MIKOLAJCZAK, C. R. SMITH, JR. and L. W. TJARKS, Northern Regional Research Lab., Peoria, Ill. 61604.

Nitrile-containing lipids have previously been isolated from *Cordia verbenacea* (Boraginaceae) and *Stocksia brahuica* (Sapindaceae) seed oils and characterized. Another sapindaceous seed oil, that of *Koelreuteria paniculata*, has now been shown to contain 42% cyanolipids, which are of two types. One of these (25% of the oil) is a mixture of diesters composed of two fatty acid moieties (predominantly C_{18} and C_{20}

monoenoic) esterified with an isoprenoid dihydrorynitrile (1-cyano-2-hydroxymethylprop-1-ene-3-ol). The other cyanolipid fraction (17% of the oil) contains monoesters comprised of a fatty acid moiety (mostly C_{20} monoenoic) esterified with 1-cyano-2-methylprop-1-ene-3-ol. These monoesters contain the same hydroxynitrile moiety as those isolated earlier from *S. brahuica* seed oil.

The structures of these cyanolipids are supported by data from GLC, IR and UV analysis, elemental analysis, NMR spectra and mass spectra. Chemical evidence confirming the structure is based largely on the hydrogenated cyanolipids since these derivatives are more stable than their unsaturated counterparts under many reaction conditions. During hydrogenation of the diesters some hydrogenolysis of ester groupings also occurred. The hydrogenated diester was reduced with lithium borohydride and the resulting dihydroxynitrile portion was isolated. Acetolysis of the hydrogenated diester in glacial acetic acid with sulfuric acid catalyst yielded an acetylated γ-lactone derived from the dihydroxynitrile moiety.

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A HYDROXY-EICOSADIENOIC ACID FROM LESQUERELLA AURICULATA SEED OIL. R. Kleiman, G. F. Spencer, F. R. Earle and A. S. Barclay, Northern Regional Research Lab., Peoria, Ill. 61604.

The seed oil of *Lesquerella auriculata* contains 32% of a previously unknown fatty acid that has been identified as 14-hydroxy-*cis*-11,*cis*-17-eicosadienoic acid; we propose for it the trivial name "auricolic acid." Its characterization involved use of GLC, TLC, IR, NMR, ozonolysis-GLC, and mass spectrometry. In addition, the oil contains 2% densipolic (12-hydroxy-*cis*-9,*cis*-15-octadecadienoic) acid, 10% lesquerolic (14-hydroxy-*cis*-11-eicosenoic) acid, and 5% ricinoleic acid. Although ricinoleic acid occurs in at least trace amounts in all *Lesquerella* oils analyzed, densipolic and lesquerolic acids have never before been reported in the same oil. The oil of *L. auriculata* is also unusual in that it includes some tetra acid glycerides. With the exception of *L. auriculata*, the analyzed seed oils of *Lesquerella* species fall into two clear-cut compositional categories which correlate with their geographic distribution; those containing densipolic acid as the characteristic component occur only in the southeastern U.S. whereas those with lesquerolic acid as the characteristic component are distributed mainly in the western U.S. It appears that *L. auriculata* whose seed oil contains lesquerolic and densipolic acids, and a third acid exhibiting structural features of both, may prove to be an evolutionary link between the two groups.

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SEPARATION AND CHARACTERIZATION OF THE BITTER COMPONENT FROM HEATED AVOCADO. M. Peled, A. Doley and A. Letan, Department of Food and Biotechnology, Technion, Haifa, Israel.

Methods of extraction and fractionation procedures are described. The effect of various chemical treatments on the bitter component were studied. It was demonstrated that formation of bitterness is not caused by heat only. Macerated avocado tissue will get bitter also when under refrigeration. The bitter component formed in heated avocado tissue was found to be a lipidic component extractable with organic solvents. The bitter compounds were separated from the lipid extract by adsorption on talc. This bitter fraction was again fractionated into 11 fractions by thin layer chromatography. Only one of the TLC fractions was found to be extremely bitter and another was on the verge of bitterness. Infrared and ultraviolet spectra of the more bitter component indicate the presence of ester groups and conjugated double bonds.

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PHOSPHOINOSITIDES OF THE SEEDS OF GOSSYPIUM HIRSUTUM. C. B. Sharma, Department of Chemistry, Marshall University, Huntington, W.Va. 25701.

The myo-inositol phospholipid fraction of the seeds of *Gossypium hirsutum* accounts for about 3.2% of the total phospholipid phosphorus which was analyzed and was shown to consist mainly of monophosphoinositide and diphosphoinoritide. Trace amounts of triphosphoinositides were also present but no evidence was found for the presence of tetraphosphoinositides. Myo-inositol phosphate components of the mono- and diphosphoinositides, liberated by base hydrolysis, were isolated and characterized. The monophosphoinositide gave myo-inositol-1-phosphate which is the same as that found for the soybean monophosphoinositide. Diphosphoinositide gave two myo-inositol diphosphates. These were characterized as myo-inositol 1,4-diphosphate and myo-inositol 4,5- or 1,6-diphosphate. Structures of myo-inositol triphosphate components of the triphosphoinositide were not determined.

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SELECTIVE BINDING OF STEROLS IN CHLOROPLASTS AND IN FUNGI. B. A. Knights, University of Glasgow, Botany Research Laboratories, Garscube Estate, Switchback Rd., Bearsden, Glasgow, Scotland.

Sterols have long been extracted from plants and fungi using light petroleum solvent. However it has been reported that sterols in chloroplasts could be divided into two fractions by successive extraction with light petroleum and then acetone, and that the second fraction contained cholesterol as the predominant sterol. Some results have been obtained confirming this observation using chloroplasts obtained from several plant sources. Some of the possibilities envisaged from these preliminary results will be discussed. Fungi such as the Ascomycete *Sordaria fimicola* are known to produce ergosterol as the principal light petroleum extractable sterol. However, using a successive extraction procedure, qualitative and quantitative differences in sterol content have been observed. These results and similar results obtained using the sterol requiring fungus *Phytophthora cactorum* will be described briefly.

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DISTRIBUTION OF STEROLS IN ALGAE. G. W. Patterson, Botany Department, University of Maryland, College Park, Md. 20740.

With the recent isolation of sterols from blue-green algae, it has been established that sterols occur in all major groups of living organisms. While in higher plants the major sterols are sitosterol, campesterol and stigmasterol, in lower plants the sterols are much more variable. In higher plants entire families commonly have a similar composition, while in algae, the sterol composition is commonly quite different even at the genus and species level. In the green algae (*Chlorophyta*), sufficient data for comparison is available in only two of the orders. The sterol composition is characteristically 28 isofucosterol with small to moderate amounts of cholesterol in the order *Ulvales*. In members of the *Chlorococcales*, there is no characteristic sterol. Members of this order may contain 28 or 29 carbon sterols with Δ^5, Δ^7 or $\Delta^{5,7}$ double bonds in the sterol nucleus. All of nearly all species of red algae (*Rhodophyta*) contain cholesterol. Some species contain desmosterol or 22-dehydrocholesterol in addition to cholesterol. The red algae have not conclusively demonstrated the ability to carry out the typical Δ^{24} alkylation reaction of other plants. The primary sterol of the brown algae (*Phaeophyta*) is fucosterol. Some species also contain small amounts of cholesterol, 24-methylene cholesterol, and saringosterol. Sterols have been identified in the other classes of algae, but too little data is available to make generalizations at this time. Further work on the sterols of algae should aid our knowledge of the evolution of sterol biosynthesis as well as our knowledge of plant evolution.

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THE INTERACTION OF STEROLS AND TETRAHYMENA PYRIFORMIS. F. B. Mallory and R. L. Conner, Department of Chemistry, Bryn Mawr College, Bryn Mawr, Pa., 19010.

Sterols are not found in *Tetrahymena pyriformis* when this protozoan is grown in a medium free of exogenous sterols; instead, the principal solid alcohol that can be isolated from the organism is tetrahymanol, a pentacyclic triterpenoid alcohol with an unusual structure. The biosynthesis of tetrahymanol has been shown by appropriate labeling studies to involve a direct, nonoxidative, proton-initiated cyclization of squalene rather than the more commonly found type of mechanism involving squalene 2,3-oxide as an intermediate. In contrast, when *Tetrahymena pyriformis* is incubated in the presence of any one of a wide variety of added sterols, the biosynthesis of tetrahymanol is inhibited, and the added sterol is accumulated by the organism and converted metabolically into one or more other sterols. Four different types of transformation have been observed: the introduction of Δ^5, Δ^7 and Δ^{22} double bonds, and the removal of C_{24} ethyl groups; for example, cholestanol, cholesterol, 7-dehydrocholesterol, 22-dehydrocholesterol and stigmasterol are all converted by *Tetrahymena pyriformis* to the previously unknown sterol, 7,22-bisdehydrocholesterol. Both in vivo and in vitro studies of the sterol inhibition of tetrahymanol biosynthesis will be described.

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TRITERPENE BIOSYNTHESIS IN ECHINODERMS. L. J. Goad, A. G. Smith and T. W. Goodwin, Department of Biochemistry, University of Liverpool, United Kingdom.

Early work showed that many invertebrates contain complex sterol mixtures. More recent studies indicate that while some species are apparently unable to synthesize sterols, others produce squalene and a few can make sterols. Among marine invertebrates echinoderms are of special interest. The Holothuroidea and Asteroidea contain Δ^7-sterols while the Ophiuroidea, Echinoidea and Crinoidea have Δ^5-sterols. In addition the Holothuroidea and Asteroidea contain toxic steroidal saponins designated holothurins and asterosaponins. Attempts to demonstrate sterol biosynthesis in the echinoid *Paracentrotus lividus* and the holothurian *Stichopus japonicus* were unsuccessful although *S. japonicus* incorporated C^{14}-acetate into squalene. The only sterol biosynthetic study on asteroids known to us is the demonstration that *Pisaster ochraceus* converts dietary cholest-5-enol into cholest-7-enol. We have therefore initiated a study of triterpene, sterol and saponin biosynthesis in echinoderms, particularly the Asteroidea. Examination showed that several asteroids contained a complex mixture of Δ^7-sterols belonging to the C_{27}, C_{28} and C_{29} series with cholest-7-enol as a major component in most cases. In *Asterias rubens* gas chromatography and mass spectrometry indicated the presence of lanosterol in the triterpene alcohol fraction. Triterpene and sterol biosynthesis was investigated by injecting [2-C^{14}] mevalonic acid into specimens of *A. rubens* and *Henricia sanguinolenta*. In both cases radioactivity was efficiently incorporated into the nonsaponfiable lipids and was predominantly distributed between the squalene and triterpene alcohol fractions. The labeled squalene was characterized by the thiourea adduct and hexahydrochloride derivative while the triterpene alcohols contained labeled components which co-chromatographed and co-crystallized with lanosteryl and dihydrolanosteryl acetates respectively. The sterol fractions contained only low levels of radioactivity and the identity of the labeled material is being investigated.

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FUNCTIONS OF STEROLS IN PLANTS. Erich Heftmann, Western Regional Research Laboratory, Albany, Calif. 94710.

Sterols have at least two functions in animals: some sterols are precursors of other steroids and some of them are hormones. It is now known that plants similarly convert sterols to other steroids, such as insect-molting hormones and other steroid hormones. It seems unlikely that steroid hormones exist in plant cells without exerting some effect on them. On the contrary, it appears reasonable to propose that the biochemical mechanisms underlying such fundamental life processes as differentiation and reproduction are analogous in plants and animals on the cellular and molecular level. The hypothesis is advanced that some sterols have hormonal functions in plants. Evidence in support of this hypothesis will be cited and approaches to testing it will be discussed.

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DETERMINATION OF SERUM TRIGLYCERIDES. R. F. Witter and Virginia S. Whitner, National Communicable Disease Center, Lipid Standardization Laboratory, Atlanta, Ga. 30333.

Over the last decade, a number of methods for the direct determination of serum triglycerides or, more properly, neutral glycerides have been developed. These methods are based on the determination of glyceride esters or, more commonly, glyceride glycerol in phospholipid free lipid extracts. In some methods, phospholipids are adsorbed after extraction of the lipids and replacement of the polar extracting solvent with a nonpolar solvent. However in most procedures, the steps of extraction of lipids and adsorption of phospholipids are combined. After either type of extraction and adsorption, glyceride glycerol is determined enzymatically or by the Skraup reaction or, more generally, by periodate oxidation to formaldehyde and subsequent determination of the latter by the color reaction of Eegriwe, Schryver or Hantzsch. Semiautomated procedures include a manual step for the combined extraction of lipids

and removal of phospholipids and other interfering compounds. Glyceride glycerol is determined automatically with Technicon equipment by means of an enzymatic reaction or, more frequently, by periodate oxidation to formaldehyde followed by Eegriwe's colorimetric or Hantzsch's fluorometric reaction. Automated saponification or transesterification is included only in those procedures in which the fluorometric Hantzsch reaction is used. Inadequate recovery of triglycerides and removal of phospholipids, carbohydrate, or other interfering compounds from the lipid extract and failure to give results in molar units are major sources of error. Recent studies indicate that precise and comparable results can be obtained under routine operating conditions with both manual and semiautomated methods.

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INFRARED SPECTROPHOTOMETRIC METHODS FOR THE ANALYSIS OF BLOOD LIPIDS. N. K. Freeman, Donner Laboratory, University of California, Berkeley, Calif. 94720.

The application of infrared spectrometry to the study of blood lipids and lipoproteins are summarized, with the aim of bringing together a body of information that is of practical use to researchers in this field. Some basic background is given in techniques as well as in the empirical interpretation of spectra. Reference spectra are included and serve to illustrate the spectral characteristics of the various lipid classes. Those features that are of particular significance in the qualitative analysis of lipids are emphasized. The fundamental aspects of quantitative analysis are outlined briefly, and some specific infrared methods are reviewed. The use of computers in connection with such analyses is also discussed.

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ISOLATION AND ANALYSIS OF HUMAN SERUM LIPOPROTEINS. F. T. Lindgren, L. C. Jensen and F. T. Hatch, Donner Laboratory, University of California, Berkeley, Calif. 94720.

A cumulative flotation preparative procedure permitting ultracentrifugal subfractionation of all major serum lipoprotein classes will be presented. This technique employs appropriate high performance swinging bucket rotors and nonlinear NaCl gradients for subfractionation of the $S_f > 400$ (chylomicron containing class), the S_f 20–400 (VLDL) and S_f0-20 (LDL) classes. An equilibrium NaBr gradient is similarly employed for the subfractionation of the high density lipoprotein class for which a special SW 45 swinging bucket rotor is needed. Total lipoprotein fractions and lipoprotein subfractions are analyzed by analytic ultracentrifugation involving a special data acquisition and control system. S_f rates, molecular weights and hydrated densities are obtained by ηF^o versus ρ procedure. Precise lipoprotein mass and protein content are determined by elemental CHN and P analysis. Where appropriate the results of ultracentrifugal lipoprotein analysis will be compared with the more common paper and agarose electrophoresis techniques.

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THE PROTEIN MOIETIES OF THE LIPOPROTEINS OF HUMAN SERUM. Virgie Shore and Bernard Shore, Lawrence Radiation Laboratory, University of California, Livermore, Calif. 94550.

Several different polypeptides have been isolated from each of the major classes of human serum lipoproteins, the high density lipoproteins (HDL, 1.065–1.195 g/ml), the low-density lipoproteins (LDL, 1.007–1.065 g/ml), and the very low-density lipoproteins (VLDL, <0.98 g/ml). The major polypeptide components of HDL, which contained carboxyl-terminal glutamine and threonine, were found in only one other fraction, the HDL_1 or 1.050–1.063 g/ml lipoproteins. Both of these peptides were about 15,000 in molecular weight but very different in amino acid composition. Increasing amounts of several other peptides were found as the density of HDL decreased. Two of these, one of which contained carboxyl-terminal alanine and the other a high percentage of serine, glutamic acid and glycine, were found in LDL and VLDL fractions of density 1.050–1.063 g/ml, 1.007–1.019 g/ml and <0.98 g/ml, but not in LDL of density 1.029–1.040 g/ml. Several different polypeptides, all less than 15,000 in molecular weight, and additional peptides present in smaller amounts were isolated from VLDL. These studies indicate that each of the major lipoprotein classes isolated on the basis of density is a mixture of lipoproteins differing in the nature of the protein moiety as well as in lipid content. The lipoproteins may contain a number of subunits since their polypeptide components are small in size in comparison with the total amount of protein in the lipoprotein molecule. Observed differences in structure among the different polypeptides suggest that some may function primarily as lipid-binders while others may serve primarily to confer water solubility on the lipoprotein molecule. HDL and VLDL and LDL fractions of density 1.050–1.063 g/ml and 1.007–1.019 g/ml may be related structurally and metabolically by their content of two common polypeptides; LDL and VLDL may be related by other polypeptides not yet isolated; HDL and LDL of density 1.029–1.040 g/ml are not related by common polypeptides.

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THE EFFECT OF ISOMERISM UPON METABOLISM OF UNSATURATED FATTY ACIDS. R. T. Holman, Hormel Institute, Austin, Minn. 55912.

Nutritional and biochemical studies of the effects of geometric and positional isomerism will be reviewed. The fate of ingested uncommon isomers will be discussed. In vitro studies of series of isomeric monoenoic and dienoic acids have revealed the importance of double bond position in governing the rates of enzymatic reaction. Data for relative rates of reaction for several enzyme systems will be presented using isomeric 18:1 and 18:2 acids as substrates, and the effects of isomeric 18:1 acids upon growth of cellular systems will be shown. Examples of effect of isomerism of polyunsaturated acids upon enzyme reactions will also be presented.

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THE DEPOSITION OF FATTY ACIDS IN THE RAT FED PARTIALLY HYDROGENATED CORN OIL. Joyce L. Beare-Rogers, Food and Drug Research Laboratories, Ottawa, Canada.

The relative nutritional roles of the various isomers of unsaturated fatty acids produced during hydrogenation have intrigued many investigators. To study the incorporation into tissue of fatty acids from liquid or partially hydrogenated corn oil, gas chromatographic results which do not distinguish polyenoic isomers were compared with lipoxidase results which show the *cis,cis*-methylene-interrupted isomers. The amount of polyenoic acids determined by the two methods was similar in the liver lipids of rats fed either the unhydrogenated or partially hydrogenated oil. A larger quantity of total fatty acids and of total polyenoic acids was found in the liver of rats fed the liquid oil than in those fed the hardened product, the difference being accounted for in the neutral lipid fraction. It appeared that the *trans* acids were mostly metabolized and that the original *cis,cis*-linoleic acid remaining in the partially hydrogenated corn oil was preferentially deposited in rat tissue.

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TRANSFER AND ADSORPTION FACTORS AFFECTING PARTIAL HYDROGENATION OF TRIGLYCERIDE OILS. L. F. Albright, School of Chemical Engineering, Purdue University, Lafayette, Ind. 47907.

The numerous transfer, adsorption and true hydrogenation steps which occur during the partial hydrogenation of triglyceride oils are reviewed and discussed. Transfer steps involve the transfer or diffusion of the unsaturated groups to the catalyst surface and possibly also into the pores of the catalyst. In addition, the reaction products must then also be transferred back to the main body of the triglyceride oil. Such reaction products include not only the saturated groups (formed by the hydrogenation of the unsaturated groups) but also the geometrical and positional isomers of the original unsaturated groups. Once an unsaturated group reaches the catalyst surface, it is generally assumed that it is adsorbed on the catalyst. Polyunsaturated fatty acid groups are however preferentially adsorbed relative to monounsaturated fatty acid groups. The overall kinetics of hydrogenation affects the relative ratio of the adsorption of the polyunsaturated to the monounsaturated groups at the catalyst surface. Transfer and adsorption steps frequently, if not always, are the critical steps in controlling the degree of isomerization and selectivity of reactions in the partial hydrogenation process. Additional information is still needed relative to these steps but the general trends which occur are discussed.

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THE EFFECTS OF HYDROGENATION PROCESS VARIABLES ON THE FORMATION OF TRANS UNSATURATION IN OILS. R. R. Allen and Jesse E. Covey, Anderson Clayton Foods, Richardson, Tex. 75080.

The amount of *trans* unsaturation formed during hydrogenation of oils depends on the temperature, pressure, catalyst concentration and agitation of the reaction. A study of the rate of formation of *trans* double bonds compared to the rate of saturation over a wide range of process conditions resulted in second degree polynomial equations. These equations may be used to calculate the conditions necessary to produce a desired amount of *trans* unsaturation at a given iodine value.

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TAILOR-MADE GLYCERIDES BY STEREOSELECTIVE HOMOGENEOUS HYDROGENATION WITH CHROMIUM TRICARBONYL COMPLEXES. E. N. Frankel, F. L. Thomas and J. C. Cowan, Northern Regional Research Laboratory, Peoria, Ill. 61604.

The preparation of synthetic fats simulating the fatty acid composition of natural, edible fats and oils has been a long-sought objective in fat chemistry. We have prepared several simulated fats by taking advantage of the unique property of chromium carbonyl complexes in catalyzing hydrogenation of polyunsaturates to *cis*-monounsaturates. The soluble $Cr(CO)_3$ complexes of methyl benzoate, benzene and cycloheptatriene gave products containing less than 10% *trans* unsaturation. Oils simulating the composition of peanut oil were prepared by hydrogenating soybean oil stereoselectively to an IV of 94. Simulated olive oil was made similarly from soybean and safflower oils hydrogenated to an IV of 82–84. Selective 1,4-reduction of eleostearate in tung oil yields a mixture of linoleate and of *trans*-9-,*cis*-12- and *cis*-10,*trans*-13-dienoates. To simulate safflower oil, conditions favoring a high proportion of linoleate were established that permitted stereoselective hydrogenation of tung oil. The unique triglyceride structure, POS (1-palmito,2-oleo,3-stearin) of cocoa butter was simulated by selectively hydrogenating linoleate in the 2 position of triglycerides in cottonseed oil stearines and in fractionated high-palmitate stearines. Presumably the POP structure obtained (where O = *cis*-monoenes) is the major component in the synthetic cocoa butter.

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SAMPLING AND MEASUREMENT IN THE PLANT. W. M. Berger, French Oil Mill Machinery Co., Piqua, Ohio.

Abstract not available at press time.

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ECONOMICS OF QUALITY CONTROL OPERATIONS. W. T. Coleman, Anderson Clayton & Co., Abilene, Tex. 79604.

Abstract not available at press time.

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QUICK PROTEIN ANALYSIS. D. C. Udy, Udy Analyzer Co., Boulder, Colo. 80302.

Abstract not available at press time.

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MEAL AND GRAIN SAMPLING. Bruce Wheaton, Carter Day Co., Minneapolis, Minn. 55418.

Abstract not available at press time.

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EXPLOSIVE GAS DETECTION AND APPLICATIONS. S. D. Delavne, Mine Safety Appliance Co., Pittsburgh, Pa.

Abstract not available at press time.

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THE METABOLISM OF CHOLESTEROL AND OTHER STEROLS BY BRAIN TISSUE. R. B. Ramsey, S. H.

MUJTABA NAQVI, J. P. JONES and H. J. NICHOLAS, St. Louis University School of Medicine, St. Louis, Mo.

Cholesterol biosynthesis in young and adult brain has been studied at the subcellular level in vitro, complementing previous work with adult brain cell-free preparations. Microsomal and supernatant fractions from adult rat brains convert MVA-2-^{14}C in excellent yield (also Na-acetate-2-^{14}C and U-^{14}C glucose in lesser yield) to ^{14}C-labeled-nonsaponifiable material, largely squalene. As with cell-free extracts the reaction is largely blocked at the squalene stage, with only *about 0.1%* of MVA converted to digitonin-precipitable sterol. Surprisingly, analogous brain preparations *from young* (10 to 15 day old) rats exhibited the same phenomenon. The defect in both cases lies *in the microsomal* preparations, since the addition of liver microsomes to brain supernatant fractions affords excellent biosynthesis of cholesterol. Neither an active adenosine triphosphatase nor a deficiency of NADPH is responsible for the in vitro defect. The isolation of lithocholic acid from EAE guinea pig brains, the ability of brain tissue *in vitro to* reduce 3-keto-5β-cholanoic acid to lithocholic acid, and the detection of *the latter acid in a specimen* of multiple sclerosis brain tissue have again raised the possibility that brain can *degrade as well as* synthesize cholesterol; this will be discussed.

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SOME PATHWAYS AND MECHANISMS IN LANOSTEROL-CHOLESTEROL CONVERSION IN MAMMALIAN TISSUES. R. PAOLETTI, G. GALLI, E. GROSSI PAOLETTI, A. FIECCHI and A. SCALA, Institute of Pharmacology, University of Milan, Italy.

Cholesterol biosynthesis in mammalian tissues takes place through various pathways; however, it is very difficult to establish their comparative importance in different tissues. In particular it is not known if the biosynthesis occurs preferentially through intermediates with or *without* unsaturation in the lateral chain. The presence of small amounts of sterol intermediates *of cholesterol* with a double bond in the lateral chain in the nervous tissue of chick embryo, rat and man has been previously demonstrated by us. Moreover, biosynthetic experiments with labeled mevalonate showed that the unsaturated intermediates are preferentially labeled when compared with the correspondent saturated sterols. These findings are particularly evident in the immature brain and in brain tumors, both tissues having *a high cholesterol biosynthetic* rate. After treatment with Triparanol (an inhibitor of the Δ^{24}-reductase) *there is an* accumulation of sterols with a double bond in the lateral chain. In the liver, the presence of dietary *sterols complicates* the picture; however, various intermediates have been detected in small amounts. Another series of investigations has been devoted to the clarification of some mechanisms in the lanosterol-cholesterol transformation. The elimination of the 15 αH of lanosterol has been already described by us and a 8,14 diene system has *been demonstrated* to be an obligatory step in cholesterol biosynthesis. Various ways of formation of *these diene systems* have been postulated, the dehydrogenation of the 8(14) ene being excluded. Other *possible mechanisms* are under investigation.

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CHOLESTEROL TURNOVER IN RATS. JACQUELINE DUPONT, Department of Food Science and Nutrition, Colorado State University, Fort Collins, Colo. 80521.

Cholesterol turnover is the sum of the processes of ingestion, biosynthesis, catabolism and excretion. The rate of turnover (or the half-life) of the sterol nucleus in the animal body varies under different conditions. The *effects* of sex, age, dietary calories and lipid on cholesterol turnover will be discussed. Rats of both sexes were *fed* high saturated fat, high polyunsaturated fat or high carbohydrate diets from weaning to *three months* of age. Half-life of 4-^{14}C-cholesterol and incorporation of ^{14}C-acetate into cholesterol were measured. The turnover of cholesterol as a function of sex and diet will be reported.

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PREPARATION OF TRITIUM-LABELED STEROLS. M. J. THOMPSON, O. W. BERNGRUBER and P. D. KLEIN, Argonne National Laboratory, Argonne, Ill. 60439.

Radiochemically labeled sterols are essential to metabolic studies and their preparation is often best achieved through collaboration of the ultimate user and the radiochemist. Among the considerations that such collaborators must face are the following: Labeling should be introduced as late as possible in the synthesis of intermediates and should require only simple structural modifications. The label, where possible, should be in locations that are known and that do not participate in undesired metabolic reactions. Radiochemical purity *should be easily attained and readily* demonstrable, while the specific activity should be adequate for the anticipated studies without excessive self-decomposition. A useful combination of these features is embodied in a procedure developed in our laboratories that is generally applicable to 3-hydroxy sterols. It consists of oxidation of the hydroxyl to a ketone, chromatography of the ketone on tritium labeled alumina to achieve exchange labeling of enolic positions, reduction and separation of the stereo isomers. Originally applied to the *preparation of ketosteroids, it has also* been used to prepare tritium-labeled 14-α methyl sterols and bile acids. We shall describe the preparation of a series of highly purified, labeled sterols including 2,4-^{3}H stigmasterol, sitosterol, fucosterol, 7-dehydrocholesterol and norcholenic acid methyl ester. Criteria of identity and purity for these products will be presented together with the yields and specific activities which have been attained. The advantages of this methods over conventional catalytic labeling techniques have been demonstrated by metabolic studies on *these products and will be reviewed.*

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RECENT STEREOCHEMICAL AND MECHANISTIC STUDIES IN STEROL BIOSYNTHESIS. G. J. SCHROEPFER, JR., B. N. LUTSKY and J. A. MARTIN, University of Illinois, Urbana, Ill. 61801.

The enzymatic conversion of lanosterol to cholesterol involves: (a) *reduction of the* Δ^{24}*-double bond*, (b) shift of the nuclear double bond from the Δ^{8}-position to the Δ^{5}-position, and (c) removal of the three extra methyl groups. Considerations of possible mechanisms of removal of carbon atom 32 of lanosterol led to the observation that $\Delta^{8(14)}$-cholesten-3β-ol is present in rat skin and is convertible to cholesterol in rat liver homogenate preparations. Stimulated by the observation by Canonica et al., of stereospecific *loss of hydrogen* from carbon atom 15 upon enzymatic conversion of lanosterol to *cholesterol, we have integrated the convertibility* of $\Delta^{8,14}$-cholestadien-3β-ol and $\Delta^{7,14}$-cholestadien-3β-ol to cholesterol. Moreover, we have investigated the metabolic fate of the hydrogen atoms at carbon atoms, 2,6,11,12,16, and 23 in the conversion of lanosterol to cholesterol. The results of recent studies of the metabolic interrelationships of $\Delta^{8(14)}$-, Δ^{8}-, Δ^{7}-, $\Delta^{8,14}$-, $\Delta^{7,14}$-, $\Delta^{5,7}$- and Δ^{5}-sterols will be summarized.

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EFFECT OF EXTRACTION PROCEDURE ON STABILITY OF SAFFLOWER OIL. A. R. KEMMERER and F. U. ROSENSTEIN, Department of Agricultural Biochemistry, University of Arizona, Tucson, Ariz. 85721.

In research previously reported no correlation was observed between oxidative stability and tocopherol contents of safflower oils containing 75% to 80% linoleic acid and 424 to 635 μg tocopherol per gram of oil. In the research reported here the solvents used *in the extraction of the safflower seeds* had considerable effect upon the oxidative stability of the oil *obtained*. Extraction with Skellysolve F gave an oil with low stability. Addition of a chloroform-methanol extract of the residue remaining after extraction with Skellysolve F definitely increased the stability. Skellysolve-ethanol extraction also produced oil with relatively high oxidative stability.

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OXIDATION AT INTERMEDIATE MOISTURE LEVELS. T. P. LABUZA, MYRON SILVER and MIRIAM SOKOLOFF, Department of Nutrition and Food Science, M.I.T., Cambridge, Mass. 02139.

Dehydrated foods at low moisture content tend to increase in their susceptibility to oxidative rancidity as the moisture content is reduced below 5–10% water. As moisture increases, stability to oxidation increases due to several known mechanisms. Currently, many *food items of the intermediate* moisture level of 30–40% water are being introduced on the *market. Based on the results* of dehydrated food oxidation, the expectation was that oxidation would not be a problem and that the major cause of deterioration would be non-enzymatic browning. Studies in model systems simulating intermediate moisture foods show, however, that oxidation of the lipid fraction is enhanced due to increased mobility of the reactants. Certain phenolic type antioxidants and chelating agents give increased stability; however, the end products of oxidation which have reducing activity increase the rate of browning if proteins are present in the systems.

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TRAPPED RADICALS IN PROTEIN MATRICES—INTERACTION WITH ADDED ANTIOXIDANTS. W. T. ROUBAL, Bureau of Commercial Fisheries, U.S. Department of the Interior, Seattle, Wash. 98102.

Ample evidence has shown lipid peroxidation to be a chain reaction process involving free radical intermediates. Nevertheless, it appears unlikely that free radicals in living systems can be easily characterized—rapid-flow EPR investigations using model systems favoring maximum radical formation have shown that only very weak resonances can be detected. Likewise, other investigators *failed to characterize* weak resonances in human serum as those resulting specifically *from lipid peroxidation; the* nature of the radical or radicals remains unknown. Thus, in living systems or in any system containing considerable amounts of water (thereby providing freedom of molecular mobility), the low steady-state radical concentration impairs radical detection and study. However, in the case of biological systems containing oxidizable lipid but freed of most water, the situation is different. Evidence shows that radicals are effectively trapped. *Furthermore, it* has been observed that addition of various antioxidants to a *system before onset of lipid oxidation*, effectively reduces the level of trapped radicals derived from immediate oxidation. EPR evidence suggests a charge migration mechanism in which unpaired electron density is transferred along protein chains to peroxy or similar radicals. Antioxidant free radicals so produced then slowly decay.

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RELATIONSHIP BETWEEN ANTIOXIDANT AND ANTI-HEMOLYTIC ACTIVITIES OF VITAMIN E DERIVATIVES. W. A. SKINNER, R. M. PARKHURST and H. L. JOHNSON, Stanford Research Institute, Menlo Park, Calif. 94025.

A number of derivatives of α-tocopherol and its model (6-hydroxy-2,2,5,7,8-pentamethylchroman) were synthesized and evaluated for their ability to protect β-carotene in hydrogenated corn oil from air oxidation. *These same derivatives were* evaluated for their ability to protect blood from vitamin E *deficient rats from hemolysis induced by dialuric acid.* Some of the model compounds were as active as the α-tocopherol *derivatives in the* in vitro blood hemolysis system used. However, as was the case with previously studied biological effects of these compounds (muscular dystrophy in rabbits and liver necrosis in rats), no perfect correlation was found relating the biological effects of these derivatives and their in vitro determined antioxidant effects.

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BIO-ASSAYS FOR ANTIOXIDANTS. S. S. EPSTEIN, Children's Cancer Research Foundation, Inc., and Harvard Medical School, Boston, Mass. 02115.

Antioxidants are widely used for a variety of industrial purposes and as food additives for stabilization of unsaturated lipids and for the prevention *of tocopherol deficiency syndromes* in animals. While chemical methods for measuring antioxidant *activity are well established*, biological methods for their estimation and characterization, in general, are perforce restricted to those few antioxidants with vitamin E biopotency. For these reasons, bioassays for antioxidants have been developed based on their ability to protect biological systems against the oxygen-dependent photodynamic toxicity of polycyclic aromatic hydrocarbons. Approximately 100 antioxidants, hindered phenols, aromatic amines, *quinolines and synergists*, were tested over a wide range of concentrations, 1 mg/ml-1 $\mu\mu$g/ml, *for their ability to protect* suspensions of protozoa or suspensions of isolated rat liver mitochondria against photosensitizing injury due to benzo[a]pyrene at a concentration of 0.1 μg/ml. Protection was shown to be highly reproducible and dose-dependent. Results in both biological systems were highly and positively correlated; these biological data were significantly but less perfectly correlated with available chemical data.

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NUTRITIONAL EFFECTS OF MILK SUBSTITUTES IN MONKEYS AND RATS. Hans Kaunitz and Jaime Sanyer, College of Physicians & Surgeons, Columbia University, New York, N.Y. 10032.

Studies on the effects of feeding skim milk filled with milk fat, coconut oil and soybean oil to monkeys and rats have been carried out for two years. In these studies, milk has supplied at least 80% of the total caloric intake. No differences in growth, appearance and milk intake have been observed. Over six months ago, a similar study was begun in which cynomolgus monkeys and weanling male rats were given an imitation milk containing 3.5% of protein from sodium caseinate and whey, 3.1% hydrogenated coconut oil, 0.75% sucrose; 5.2% lactose and salts, and 0.15% monoglyceride emulsifier. At present, both species show no differences as to appearance, growth, milk intake and appearance of stools in comparison to their controls fed filled milk.

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FATTY ACID COMPOSITIONAL STUDIES OF CHILDREN IN NORMAL AND ABNORMAL NUTRITIONAL STATES. J. R. Paulsrud, R. T. Holman, C. F. Whitten, Leslie Pensler and William Krivet, The Hormel Institute, Austin, Minn. 55912.

The fatty acid composition of triglycerides, nonesterified fatty acids, cholesterol esters and total phospholipids from the sera of normal children will be compared with those suffering from acrodermatitis enteropathica (AE) and "failure to thrive." The patients suffering from AE have been sampled throughout the course of treatment which involved the use of intravenous lipid infusion. Those suffering from failure to thrive have been followed from admission, through treatment with lipid-free infusions of essential nutrients, to discharge as "well" infants. The data will be discussed in terms of what is known regarding essential fatty acid requirements during the first 24 months of life.

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SOME EFFECTS OF EXOGENOUS CHOLESTEROL AND SATURATED FATTY ACIDS ON LIPEMIA. Antanas Butkus, L. Allen Ehrhart, Lena A. Lewis, A. L. Robertson and F. Merlin Bumpus, Cleveland Clinic Foundation, Cleveland, Ohio 44106.

A semi-synthetic diet A, containing 17% hydrogenated coconut oil and 5% cholesterol or a diet C identical to A, but without cholesterol supplement was fed to dogs for four months to determine the effects of exogenous cholesterol addition on lipemia produced by diets high in saturated fatty acids (FA) and lacking essential FA. Two control samples of blood were drawn from each dog before starting special diet A or C and one sample after 1,2,4,10 and 16 weeks on diet. Total cholesterol (TC), free cholesterol (FC), triglyceride (TG), phospholipid (PL) and FA of cholesterol ester (CE), TG and PL were measured. The appearance of circulating lipemia was very similar in all experimental animals: Dogs on diet A and C showed significant increases in lipid concentration and changes in FA distribution (per cent composition) within the first week on diet as compared to controls. For the first month there was no difference in TC,FC,TG,PL,CEFA,TGFA, free FA and PLFA concentration between diet A and C or FA distribution in all lipid fractions studied. At the 10th and 16th week periods serum TC,FC and PL were significantly higher in diet A than in C, but no difference in TG concentration between these two diet groups. At the latter periods some significant changes in FA distribution were seen between plasma of diet A and C dogs, e.g., cholesterol oleate was higher and cholesterol arachidonate lower with diet A. The hyperlipemia produced is not a simple reflection of these special diets as evidenced by the increase in some FA, e.g., C_{16}, $C_{18:1}$ and $C_{20:3}$, which were absent in diet. Saturated FA alone appear to be as effective in initiation of hyperlipemia as in combination with 5% cholesterol. Linear regression analysis shows that FA, except $C_{20:3}$ and $C_{20:4}$ in CE fraction correlate stronger to cholesterol concentration than to FA in TG or PL fractions. Based on correlation coefficients a hypothesis is advanced that FA esterify preferentially to cholesterol compared to TG or PL for transport. Data also suggest that exogenous cholesterol is necessary for sustaining hyperlipemia.

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URINARY METABOLITES OF RETINOIC ACID IN THE RAT. P. R. Sundaresan and H. N. Bhagavan, Lipids Laboratory, St. Joseph Hospital, Lancaster, Pa. 17604.

Retinoic acid, a derivative of retinol (vitamin A) has been shown to be biologically active in the rat except in vision and reproduction. It has been hypothesized that retinoic acid is the precursor of, or is itself a systemically active form of retinol. A previous report indicated that when specifically labeled retinoic acid was administered to rats, the decarboxylation was rapid at C_{15}, much less at C_{14} and negligible at C_8 and C_7. In the present study, groups of retinol-deficient rats were injected with equal physiological doses of 15-^{14}C, 14-^{14}C or 6,7-$^{14}C_2$ retinoic acid, and urine was collected for 24 hr. Three fractions were obtained from the urine, viz. ether-soluble, acidic and water-soluble. While the data on the distribution of radioactivity in these fractions suggest several metabolites of retinoic acid, preliminary results indicate that one of the metabolites lacks C_{14} and C_{15} of retinoic acid. The isolation of these metabolites and their significance with reference to the metabolism of retinol will be discussed.

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NUTRITIONAL STUDIES WITH CYCLOPROPENOIDS INACTIVATED WITH FATTY ACIDS. D. T. Hopkins, R. R. Dahlgren, A. W. Munson and H. P. Dupuy, Ralston Purina Company, St. Louis, Mo. 63199.

Cyclopropenoid fatty acids inactivated with fatty acids were produced by reacting *Sterculia foetida* oil (approximately 50% cyclopropenoids) with cottonseed oil fatty acids. The final product contained 35% inactivated cyclopropenoids by calculation and was negative to the Halphen test. The Halphen-negative *Sterculia foetida* oil was fed at dietary levels of 0%, 0.14%, 0.71% and 3.57% to growing rats and laying hens for four weeks. All animals were autopsied and examined microscopically for pathological lesions at the termination of the experiment. Blood hemoglobin, packed cell volume and plasma cholesterol were similar in animals fed all of the diets. No abnormal physiological response was seen in the animals that could be related to dietary treatments. Growth rate of rats and egg production of hens of all groups of animals was normal. After three and six months' storage, eggs from hens fed the Halphen-negative oil were normal and showed no evidence of the abnormalities that usually accompany cyclopropenoid feeding such as pink albumins and abnormal ratio of saturated to unsaturated fatty acids. Lipids of heart, liver and adipose tissues of rats and hens were examined for unusual fatty acid composition. Fatty acid composition was generally normal except that small amounts of three unidentified fatty acids with equivalent chain lengths between 23 and 26 were found in rats fed the two higher levels of the oil. The results of these feeding studies indicate that inactivation of cyclopropenoids with fatty acids negates the toxic effect of cyclopropenoids.

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EFFECT OF COMMERCIAL DIETS ON THE FATTY ACID COMPOSITION OF DEVELOPING SALMON. J. B. Saddler and L. S. Smith, College of Fisheries, University of Washington, Seattle, Wash. 98105.

Commercially prepared salmon diets frequently contain lipids from various animal and plant sources. When these diets are fed to developing salmon, the total lipid percentage and the component fatty acids differ markedly from the component fatty acids found in developing salmon in their native environment. Juvenile salmon fed a diet containing 20% 18:2 will retain an average percentage of 15% 18:2 in their total lipids. When the percentage of 18:2 in the diet was increased to 40%, the percentage of 18:2 retained by the fish was 30%. Salmon developing in their native habitat retain between 1% and 2% of 18:2 in their tissues. The major long-chain polyunsaturated fatty acids found in salmon fed commercial diets averaged 11% while the major polyunsaturated fatty acids found in native salmon averaged 35%. The advantage or disadvantage of the increased percentage of 18:2 in the tissue lipids on the survival of hatchery salmon is not known. Salmon fed commercial diets and released prior to their downstream migration do not retain the increased percentage of 18:2 and within 10 days following their release contain only 3% to 4% of 18:2 in their tissues.

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PHOSPHOLIPID COMPOSITION OF THE BOLL WEEVIL, ANTHONOMUS GRANDIS BOHEMAN. Rodger Henson, A. C. Thompson, R. C. Gueldner, M. Petty and P. A. Hedin, Boll Weevil Research Laboratory, State College, Miss. 39762.

The phospholipids of newly-emerged adults of the boll weevil, *Anthonomus grandis* Boheman, were studied in detail. Phosphatidyl choline was the major phospholipid at 37.5%. Phosphatidyl ethanolamine comprised 32.8% and sphingomeylin 8.9%. Three minor components are present and have been tentatively identified as phosphatidyl inositol, phosphatidyl serine and polyglycerol phosphatide. Fatty acid analyses of the intact phospholipids were performed, as well as analyses of the positional distribution of the fatty acids of phosphatidyl choline and phosphatidyl ethanolamine. Oleic acid is the major fatty acid present in the glycerophosphatides. Sphingomeylin contains fatty acids in the range of 20–22 carbons.