# European Club of Centers for Lipid Research

# Report from Belgium: The Institut de Fermentation-Meurice-Chimie, CERIA, 1070 Brussels

The Institut de Fermentation-Meurice-Chimie was founded 80 years ago and is now part of the Centre d'Enseignement et de Recherches des Industries Alimentaires et Chimiques (CERIA), a unit set up by the Province of Brabant. The purpose of this center is to organize, develop, centralize and coordinate vocational and higher

technical training for the industries of food, fermentation,

chemistry, and related branches.

The Institut de Fermentation-Meurice-Chimie is a high school for engineers in chemistry and biochemistry. Training is subdivided into two sections: Biochemical Industries, including the specializations of Fermentation Industries, Food Industries, and Applied Biological Chemistry; and Chemical Industries, including the specializations of Organic Chemistry—High Polymers, Inorganic Chemistry—Metallurgy, and Paints and Varnishes. A diploma of Technical Engineer is delivered after 4 years of training. The population of the institute is ca. 300 students and ca. 150 members of the teaching, scientific and technical staff.

Teaching activities are coordinated with fundamental and applied research, which carried out on a large scale. Research activities aim both to adapt the training to the exigencies of modern demands and to keep it in close contact with the industrial branches for which the graduates are trained.

Research is coordinated within the different departments of the institute by the CERIA Committee for the Promotion of Scientific Research. This committee is entitled to receive funds from public organizations or private industry and supervises the financial and scientific basis of the research contracts.

In the Department of Food Technology of the Institut de Fermentation-Meurice-Chimie, lipid research is conducted according to the following guidelines.

## Assessment of quality criteria in lipids allowing forecast of the stability of manufactured products from the characteristics of the crude fats and oils

- 1) Investigations on fats in biscuits: A research program is carried out for IBAN (Institut Belge de l'Alimentation et de la Nutrition), which is an association of private and public research centers. IBAN is sponsored by the Ministry of Health and was created to improve knowledge of human and animal biochemistry and physiopathology.
- 2) Analytical figures of palm oil samples drawn at different stages during the transport from the production centers to the consumer have been submitted to a multiple correlation program to study the influence of the combination of the analytical results on the estimate of oxidative depreciation. This study will lead eventually to the establishment of quality levels of acceptability. A similar investigation of olive oil samples from the Mediterranean area also gave an improved appreciation of quality.

### Monitoring of lipid texture

1) Preparation of copper-containing catalysts on supports of various porosities for selective hydrogenation of fatty acids: It could be shown that the copper-nickel catalysts produce rapid hydrogenation with satisfactory



Headquarters of CERIA, of which the Institut de Fermentation-Meurice-Chimie is a part.

yields. Cobalt reduces the *trans*-isomer content, but the total yield is decreased. The support itself influences the reaction to the same extent as the catalyst, both for selectivity and reaction rate.

- 2) Transesterification: Tributyrin and triacetin could be transesterified for any desired ratio of mono- and diacetyl triglyceride. The reaction kinetics are very intricate. The reaction is mainly of order zero; however the reaction rate may be changed by varying the concentration of the substrate. Observation under the microscope of the triglyceride-catalyst interface showed evidence of nucleation phenomena.
- 3) Several studies of optimization of formulations of food mixtures have been carried out on request of different food manufacturers, especially in the oil and fat branch.
- 4) Crystallization studies on the kinetics of palm oil crystallization have been initiated. They will be carried on in collaboration with M. Loncin and his team at the Institut für Lebensmitteltechnologie und Verarbeitung, University of Karlsruhe, Germany.

### Studies on diffusion

These investigations carried out with a diffusion cell have led to the determination of: the diffusion constant of water in glyceridic oils; the diffusion constant of surface active agents in relation to their concentration; the influence of dissolved gases on the diffusion of oxygen in lipids.

# Equilibrium relative humidity (ERH)

The influence of the ERH on the kinetics of the hydrolysis of fats has been examined. Previous studies showed that these kinetics follow a reaction order zero. The splitting rate is reduced in a rate directly proportional to the ERH. The sorption curves of fats and the influence of the temperature were assessed. Studies of the heat resistance of lipases in partly dehydrated medium have led to the set-up of stabilization of palm kernels during storage, on an industrial scale.

# Production of yeast from lipids

The conditions of the assimilation of palm oil by microorganisms have been established. The aim of this (Continued on page 354A)

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which could not be accounted for by an aggregation of microsomes and mitochondria or an exchange with microsomes contaminating the mitochondria. Under similar circumstances there was a transfer of phospholipid from <sup>32</sup>P-labelled mitochondria to microsomes, indicating that the process was one of exchange. All of the isolated individual synaptosomal membranes were capable of acquiring phospholipid on incubation with a <sup>32</sup>P-labelled brain supernatant fraction although a greater percentage was again exchanged by the mitochondrial fraction.

FATTY ACID SYNTHETASE OF CHICKEN LIVER. REVERSIBLE DISSOCIATION INTO TWO NONIDENTICAL SUBCOMPLEXES OF SIMILAR SIZE. S. Yun and R.Y. Hsu (Dept. of Biochem., State Univ. of New York, Upstate Med. Center, Syracuse, N.Y. 13210). J. Biol. Chem. 247, 2689-98 (1972). The synthetic reaction catalyzed by the chicken liver fatty acid synthetase has a bell-shaped pH rate profile with a maximum at pH 6.7 and gives a discontinuous biphasic Arrhenius plot. The activation energy at low temperature is 28,900 cal per mole, which changes to 11,300 cal per mole at temperatures above 15C.

EFFECT OF PHOSPHOLIPASE C HYDROLYSIS OF MEMBRANE PHOSPHOLIPIDS ON MEMBRANOUS ENZYMES. R.D. Mavis, R.M. Bell and P.R. Vagelos (Dept. of Biol. Chem., Washington Univ. Schl. of Med., St. Louis, Mo. 63110). J. Biol. Chem. 247, 2835-41 (1972). The response of several Escherichia coli membranous enzymes to hydrolysis of up to 95% of membrane phospholipid has been investigated. Purified phospholipase C of Bacillus cereus was utilized in these studies. The rate and extent of digestion of E. coli phospholipids were independent of whether the lipid was associated with membrane protein or extracted from membranes and sonically dispersed. Phosphatidylethanolamine and phosphatidylelycerol were completely hydrolyzed, while cardiolipin was partially resistant to hydrolysis by phospholipase C. Acyl-CoA: glycerol 3-phosphate acyltransferase and NADH oxidase were inactivated at a rate very similar to the rate of hydrolysis of total lipids. These results show the heterogeneity of membranous enzymes with respect to their dependence upon the presence of intact membrane phospholipids.

CHARACTERIZATION AND QUANTITATION OF THE APOLIPOPROTEINS FROM HUMAN CHYLE CHYLOMICRONS. G. Kostner and A. Holasek (Inst. of Physiological Chem., Univ. of Graz, Graz, Austria). Biochemistry 11, 1217-23 (1972). The composition of human chyle chylomicrons was studied. For this purpose, thoracic duct chylomicrons from a female subject were isolated, delipidized and the lipid and protein part investigated separately. For the characterization of the protein part, polyacrylamide gel electrophoresis, immunoelectrophoresis and immunodiffusion were performed. Pure peptides isolated from human serum lipoproteins were used as reference substances. The results indicate that all the peptides of human serum very low density lipoproteins were present in chyle chylomicrons too.

VITAMIN A TRANSPORT IN RAT PLASMA. ISOLATION AND CHARACTERIZATION OF RETINOL-BINDING PROTEIN. Y. Muto and D.S. Goodman (Dept. of Med., Columbia Univ. College of Physicians

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investigation was to find a cheap source of proteins, to valorize waste products from the palm oil mills, and to offer an interesting solution for pollution problems in the palm oil producing countries. The investigations have been carried out on laboratory scale with a *Candida lipolytica* strain (BP, Lavera).

The oxidative degradation of fatty acids by Candida lipolytica and tropicalis has also been studied. A significant specificity for long chain fatty acids was observed ( $C_{12}$  to  $C_{18}$ ).  $C_4$  to  $C_{10}$  fatty acid chains are not metabolized, and, moreover,  $C_8$  and  $C_{10}$  chains exert an inhibitive action.

At the Institut de Recherches of CERIA the regulation of the biological control of the catabolism of fatty acids was studied with *Escherichia coli*. Also the effects of the perturbation of the control factors on the physiology of the bacterial cell were studied.

and Surgeons, New York, N.Y. 10032). J. Biol. Chem. 247, 2533-41 (1972). Studies were conducted to isolate and characterize rat serum retinol-binding protein (RBP), the specific transport protein for vitamin A in the rat. RBP was isolated from rat serum by a sequence of procedures which included: precipitation with ammonium sulfate between 30 and 50% saturation; chromatography on DEAE-Sephadex; gel filtration on Sephadex G-200 and G-100; and preparative polyacrylamide gel electrophoresis. These procedures resulted in RBP which had been purified approximately 2,300-fold, and which was completely pure by physical and by immunological criteria. The properties of rat RBP resemble those of human plasma RBP in many ways. The two proteins have nearly identical ultraviolet absorption spectra (peak maxima at 280 and 330 nm) and fluorescence emission and excitation spectra. The amino acid compositions of rat and human RBP are somewhat similar, both with a fairly high content of aromatic amino acids.

THE EFFECT OF DIETARY SOYBEAN OIL ON THE DEPOSITION OF XANTHOPHYLL IN BROILER SKIN. J.L. Heath and C.S. Shaffner (Dept. of Poultry Sci., Univ. of Md., College Pk., Md. 20742). Poultry Sci. 51, 502-6 (1972). Two trials were conducted to determine if varying dietary levels of oil would affect xanthophyll deposition in broiler skin. The birds in both trials were fed either 4, 7 or 10% oil in the diet which provided 2.17, 2.35 and 2.53 Cal. M.E./mg, of xanthophyll, respectively. These rations were fed ad libitum from one day of age until they were sacrificed at 8 weeks of age for birds in trial 1 and 7 or 8 weeks of age for those in trial 2. Skin samples were taken from the breast and back of each bird, extracted with acetone and the xanthophyll content determined. The lipid from each sample was extracted with a chloroform-methanol mixture and weighed. The increase in dietary oil in the rations of this experiment did result in significant increases in xanthophyll deposition in the back skin for both 7 and 8 week old birds. More xanthophyll was deposited per gram of tissue as the percentage of dietary oil increased. The carcass weight and tissue lipid also increased as dietary oils were increased.

The interactions between dietary saponin, cholesterol and related sterols in the chick. B. Morgan, Monique Heald, S.G. Brooks, J.L. Tee and J. Green (Beecham Res. Labs., Nutr. Res. Center, Walton Oaks, Tadworth, Surrey, England). Poultry Sci. 51, 677-82 (1972). One week-old chicks were fed a basal semi-synthetic diet, with and without cholesterol (0.5%), for 3 weeks and the effects of digitonin (0.25%) and Gypsophila saponin (0.25%) on growth and serum cholesterol were examined. Digitonin and Gypsophila saponin both depressed growth and the depression was reversed to a large extent, but not totally, by dietary cholesterol. These two saponins lowered serum cholesterol in chicks fed either the basal diet or the cholesterol-containing diet. Chicks fed the cholesterol-containing diet supplemented with digitonin or Gypsophila saponin were dosed orally with cholesterol-4. Cheither of the saponins affected the amount of radioactivity found in the lipids of liver or serum 3 or 21 hr. after dosing. Neither 3β-hydroxy-5β-cholestanol nor 3α-hydroxy-5β-cholestanol reversed the growth depression caused by digitonin or Gypsophila saponin. These results are discussed in the light of the suggestion that cholesterol complexes with saponins in the gastro-intestinal tract.

Failure of vitamin supplementation to alter the fatty liver syndrome caused by aflatoxin. P.B. Hamilton and J.D. Garlich (Dept. of Poultry Sci., N.C. State Univ., Raleigh, N.C. 27607). Poultry Sci. 51, 688-92 (1972). A mixture of choline, inositol, vitamin  $B_{12}$  and vitamin E has been reported to cure the fatty liver syndrome of laying hens, but the inability to reproduce this syndrome in the laboratory has prevented controlled evaluation of the proposed cure. Since dietary aflatoxin causes reproducibly in the laboratory a fatty liver syndrome, an experiment was designed to establish whether the vitamin mixture was effective in treating the syndrome caused by aflatoxin. Hens selected for similar age, weight, and egg production were used in a  $2\times 2$  factorial experiment for the presence and absence of the added dietary vitamins and  $10~\mu g/g$  aflatoxin. After three weeks the aflatoxin caused a significant increase in liver size, liver total lipid and percent of egg as shell and a decrease in egg production while the added vitamins were without effect on these parameters. Half of the birds which had received aflatoxins were placed on the control diet while the other half were fed the control diet supplemented with the vitamin mixture. In three weeks, all parameters returned to control