hard fat; the crystal structure is in the *beta* form. While some of these emulsions are undoubtedly complex structures, all three forms disperse in water.

As long as the temperature is maintained above the gel point, Type A emulsions appear to be stable indefinitely. The stability of Type B emulsions varies depending upon the composition, method of preparation, and temperature of storage but may be between minutes and months. Type C emulsions appear to be stable indefinitely, and the water can be evaporated to yield a very fine, free-flowing powder. Type A emulsions can be regenerated from either Type B or Type C by heating above the gel point. This cycle is shown in Figure 3.

Effective co-emulsifiers are the strongly hydrophilic types, which are usually water-soluble, and may be anionic, nonionic, or cationic. Examples which have been used successfully include ordinary sodium soaps, sodium lauryl sulfate, polyoxy compounds (Tween S0), sucrose monopalmitate, quaternaries, and certain protein hydrolysates. The more hydrophilic materials are more effective, and less is required to accomplish the same result. Table V shows that an optimum quantity of co-emulsifier exists, at least when sodium oleate is the co-emulsifier.

TABLE V Type B Emulsion a Stability of Distilled Monoglycerides from Hydrogenated Lard with Sodium Oleate

Sodium oleate α.	Time (days) to develop β -crystals at 25°C.
0.2	
0,4	
0.5	28
0.7	
0.9	
2.0	

Summary

When concentrated monoglycerides (such as distilled products) are heated to about their melting point with water, a gel is formed. The exact temperature of gelation is dependent upon molecular weight of the fatty acid (monolaurin does not gel, but monopalmitin does) and upon the the purity of the monoglyceride.

Additives can prevent gelation, with triglycerides (15% to 20% required) about twice as effective as diglycerides (30% to 40% required). Highly hydrophilic co-emulsifiers prevent gelation, resulting in one of three types of emulsions, at least two of which are thixotropic.

Water is partially soluble in monoglycerides and in monoglyceride containing blends. By this technique many water-soluble materials can be incorporated into an oil solution.

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Oxidized Fatty Acid-Protein Complexes¹

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ROTEINS ARE KNOWN to form complexes with a wide variety of substances, such as alkyl benzene sulfonates (1), p-amino azo benzene (2), gossypol (3), phospholipides (4), sterols (5), and various other lipide materials. However most of these complexes are labile and could be easily ruptured to give back the constituent molecules in their original form by use of simple techniques, such as extraction with a suitable solvent system. Although considerable work has been done on lipoprotein complexes, very little is known regarding the structure of these conjugated proteins (6). Dervichian considered the asso-

ciation between the lipide and the protein to involve a weak lipide-lipide linkage, which could be split by ether when the water barrier was removed, and a somewhat stronger lipide-protein linkage that could be split by use of boiling alcohol (7). The presence of a strongly bound lipoprotein which is resistant to solvent action has been reported by Folch *et al.* as occurring in brain tissues (8).

Recently Tappel observed that unsaturated fatty acids and esters could react with proteins forming rather stable complexes which could not be ruptured by solvent treatments (9). He attributed the stability of these complexes as being caused by a chemical union between the aldehydes produced during autoxidation of the lipide and the reactive amino groups of the protein. Tappel obtained these stable lipide-pro-

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tein complexes by agitating a $\frac{1}{2}\%$ solution of the protein in a water or buffer medium with a large excess of the lipide in the presence of a hematin catalyst at 37°C., using a mechanical shaker. However reproducible results could not be obtained under these conditions. In the present investigation stable complexes were formed between egg albumin and oxidized linoleic acid under standardized conditions which gave rise to reproducible results. It will be apparent from the data presented that there is little likelihood of an aldehyde-amine condensation reaction taking place in the reactions leading to the complex formation. From these and other studies on the physical and chemical properties a possible structure has been proposed for these complexes.

Experimental

Formation of Oxidized Linoleic Acid-Egg Albu*min Complexes. ~* Ten g. of powdered egg albumin were dissolved in 1,000 ml. of distilled water with the aid of a mechanical stirrer. Ten g. of freshly prepared linoleie acid (iodine value 180.1, peroxide value 0.0) were added and emulsified into the protein solution. The flask containing this mixture was placed in an oil bath, and the stirring was continued at a rapid rate. The internal temperature of the lipide-protein emulsion was maintained at 60 \pm 2°C. by adjusting the temperature of the oil bath. After the specified reaction time the denatured protein-Iinoleic acid complex was obtained by filtration, soaked in acetone for 24 hrs., and extracted in a Soxhlet apparatus, first with acetone for 24 hrs., followed by ethyl ether for 24 hrs. The product was dried at 50° C. in a vacuum oven and weighed.

Three g. of the product were hydrolyzed on a steam bath for 10 hrs. with the aid of a 10% solution of aqueous potassium hydroxide. The resulting solution was acidified with hydrochloric acid and extracted with ethyl ether. The residue obtained upon evaporation of the ether extract was taken as indicative of the amount of unsaturated fatty acid complexed with the protein.³ The unreacted, autoxidized linoleic acid was obtained from the cold acetone extract by evaporation of the solvent and was freed from the last traces of acetone by water washing of the ether extract. The iodine number determinations were conducted on the reduced samples, and the acid numbers were determined by a slight modification of the official method (10). The peroxide numbers were determined according to the method of Wheeler (11) , and the carbonyl values were determined by a modification of the method described by Lappin and Clark (12). The infrared spectra were determined, using a Beckman IR 2 spectrophotometer.

Alumina-Lipide Complexes. These complexes were prepared by mixing, with a spatula, 100 g. of activated alumina with 25 g. of the lipide for 10 min. The free lipide was removed first, by addition of ethyl ether and decantation of the extract, and second, by solvent extraction of the alumina-lipide complex in a Soxhlet apparatus with ethyl ether for 48 hrs., followed by an extraction with $1,4$ -dioxane for 24 hrs. The nonextractable lipide in combination with the alumina was obtained by treatment with strong aqueous potassium hydroxide, followed by acidification and extraction with ethyl ether.

Estimation of Reactive Groups in the Protein. The reactive hydrogen groups in the protein and the protein of the protein-linoleic acid complexes were estimated by reacting them with lauroyl chloride. Three g. of the protein or the lipide-protein complex were suspended in 100 ml. of anhydrous ethyl ether cooled to 0°C. and kept stirred by means of a magnetic stirrer: Ten g. of lauroyl chloride were added rapidly, followed by the gradual addition of 35 ml. of pyridine. The reaction was allowed to proceed for three days at room temperature. After this period 20 ml. of methanol were added, and the stirring was continued for three more hours. The lauroylated product was obtained by filtration, followed by washing with ethyl ether and extraction in a Soxhlet apparatus with ethyl ether for 24 hrs. It was dried at 50°C. under vacuum and weighed. The number of lauroyl groups introduced was estimated by alkali hydrolysis, followed by acidification with hydrochloric acid, ether extraction, and gravimetric determination of laurie acid. In the case of the lauroylated protein-fatty acid complex the difference between this amount and the amount of fatty acids initially present in the complex was used for determining the number of lauroyl groups.

Results and Discussion

A saturated fatty acid like lauric acid did not complex with egg alubmin while a monounsaturated fatty acid like oleic acid reacted with egg albumin only to

^a Using 10 g. of gg albumin, 30 g. of the fatty acid, 1,000 ml. of
distilled water at 60°C.
^bProduct/initial protein means yield of the product expressed as per-
centage of the initial dry protein.
 $\frac{1}{2}$ and $\frac{1$

a very small extent (Table I). The results seemed to indicate that linoleie acid could not complex with egg albumin unless it was partially oxidized (Table II). Oleic acid and laurie acid did not possess the necessary functional groups required for complexing with proteins. These functional groups were apparently present in autoxidized linoleic acid, therefore with increasing autoxidation a corresponding increase in the weight of the denatured protein-fatty acid complex and an increase in the amount of the complexed fatty acid were obtained. However, even after a long reaction time, the amount of complexed linoleic acid in the product could not be increased to more than 8.0%.

There seemed to be no definite relationship between the number of hydroperoxide groups in the unreacted linoleic acid and the extent of complex formation (Table II). Although there seemed to be some connection between the amonnt of carbonyl groups in the unreacted fatty acid and the reactions leading to the complex formation, the decrease in unsaturation

² The conditions were chosen after preliminary investigation of **the** factors influencing the complex formation. These data win be pre-sented **in** a separate paper.

s Under similar conditions egg albumin gave rise to 0.8% of extract-able material.

$_{\rm No.}^{\rm Expt.}$	Time	Product. initial protein	Lipide, product	Unreacted lipide			Lipide material from complex				
				$_{\rm Acid}$ No.	${\bf Iodine}$ No.	Peroxide No.	Carbonyl value	$_{\rm Acid}$ No.	Iodine No.	Peroxide No.	Carbonyl value
	hrs.	%	%								
	14	68.9 81.4	0.3 3,1	194.5 164.8	170.2 107.0	166.1 741.2	0.12 1.19	191.6 253.0	69.4		0.59
	31 216	89.9 96.7	5.9 8.0	164.2 160.7	93.7 74.0	215.8 25.2	1.43 1.35	278.2 256.0	52.7 50.3		0.65 0.49

TAB LE II The Effect of the Extent of Auto-oxidation of Linoleie Acid on Complex Formation a

seemed to have a direct bearing on complex formation. This decrease in unsaturation of the autoxidized fatty acid was also attended by a corresponding decrease in the acid number, which indicated that the oxidative polymers formed were probably linked through oxygen groupings. This is in conformity with the results obtained by Chang and Kummerow (13), who observed that the oxidative dimer and trimer of ethyl linoleate had 8 and 12 extra atoms of oxygen per molecule of polymer, respectively. AIthough the infrared spectra of the unreacted linoleic acids exhibited the $O-H\cdots O$ association band near 3.7 microns, the broad band around 10.6 microns indicative of OH vibration in and out of the plane of the-COOH group was found to decrease progressively as the autoxidation proceeded (Table II).

The chemical properties of the material obtained by hydrolysis of the complexes did not in any way help to explain the nature of bonding between the lipide and the protein. The iodine and carbonyl values of these materials were found to. have dropped considerably when compared with the values obtained from the unreacted linoleic acids isolated from each experiment. In spite of the fact that these materials were fairly viscous, the acid numbers were rather high. The infrared spectra exhibited all the characteristic absorption bands of fatty acids, the C-H stretching near 3.4 microns, the $O-H \cdots O$ association band at 3.7 microns, the C=O stretching near 5.8 microns, the C-H bending around 7.0 microns, and the CH2 wagging mode near 13.7 microns, with the exception of the broad band at 10.6 microns.

TABLE III A Comparison of the Results Obtained with Various Color Reactions Which Are Applicable to Proteins

Expt. No.	Material	Amount оf lipide	Ninhv- drin	Millon's	Biuret
		%			
	Egg albumin				
$\overline{2}$	Egg albumin-linoleic acid complex	0.3		$\, +$	
3	Egg albumin-linoleic acid				
4	complex Egg albumin-linoleic acid	3.1			
	complex	5.9		┿	
5	Egg albumin-linoleic acid complex	8.0			
6	Lauroylated egg albumin-				
	linoleic acid complex	32.7			
	Lauroylated egg albumin	26.8			

The various color tests for proteins were applied to these complexes in order to determine qualitatively whether any of the reactive groups were tied up (Table III). Tappel has indicated that none of the complexes prepared by him gave the biuret or ninhydrin test. All the linoleic acid-egg albumin complexes prepared in this study gave a positive biuret and a positive Millon's test. The ninhydrin test was however negative in the case of three of the complexes. The lauroylated complex gave a positive biuret but a negative ninhydrin and a negative Millon's test.

It has been suggested that aldehydes produced during the oxidation of the unsaturated fatty acids could react with the amino groups of the protein by a type of aldehyde-amine condensation reaction (9). To investigate this possibility three aldehydes of varying chain length were reacted under the same conditions that would exist if these or other aldehydes were formed through oxidation of the unsaturated fatty acid during the course of the reaction between the lipide and the protein (Table IV). Denatured protein

a Using 10 g. of egg albumin, 1,000 ml. of distilled water and 30 g. of aldehyde at 60°C. b After alkali hydrolysis and acidification.

similar to those observed using linoleic acid were obtained. However the amount of alkali hydrolyzabte material in these products was found to be less than 2%. In the reaction with lanryl aldehyde the unreacted material was isolated and its acid number was determined. This value was roughly one-half of what would be expected if all the aldehyde had been oxidized to the acid. It would appear from these results that an aldehyde-amine condensation reaction does not take place in lipide-protein complexes. This was confirmed by the fact that lauroyl chloride was found to react with the linoleic acid-egg albumin complex to the same extent as the denatured egg albumin (Table V). The native egg albumin was found to be unreactive, which seemed to suggest that the reactive groups in the protein became available only on denaturation.

The extent of complexing with alumina and various oxidized or polymerized and fresh lipides indicated

TABLE V Estimation of the Number of AcyI Groups Introduced into the Protein and the Complex

	State of protein		Acylated product	Mole			
Protein		Reagent	Total $ext{ract}$	Polymeric lipide	acyl group ^a		
			%				
Egg albumin	Native	Laurovl chloride	0.8	0	0		
Egg albumin	Denatured by oleic acid	Laurovi chloride	27.6	0	0.186		
Egg albumin- linoleic acid	Denatured	Lauroyl					
complex		chloride	33.5	8.0	0.183		

a 100 g. of protein.

that a strong adsorption could be produced even by a simple mechanical mixing process if the necessary group or groups were present (Table VI). It is conceivable that these results could very well explain the observations made by several workers (14, 15) concerning material losses while using chromatography as a technique for the resolution of mixtures

~'resh oleic acid Alumina **-- -- 0.5** a Determined by treatment with strong aqueous alkali, as in the case of lipide-protein complexes. Washed repeatedly with ethyl ether. e Extracted in a Soxhlet apparatus with ethyl ether for 48 hrs. a Extracted in a Soxhlet apparatus with 1,4-dioxane for 24 hrs. after an initial 48-hr. extraction with ether.

of lipides or amino acids. The fact that oxidized corn oil and oxidized linoleic acid were strongly adsorbed by alumina indicated that they possessed certain functional groups that were not present in fresh corn oil or thermally polymerized corn oil. Although the present study indicated that pure linoleie acid was also strongly adsorbed, it seemed likely that the unsaturated fatty acid was oxidized on the surface of the adsorbent, which would therefore account for the irreversible adsorption. This was confirmed by the fact that oleie acid was found to be adsorbed to a much smaller extent. The small adsorption observed with oleic acid further indicated that some group or groups besides the carboxyl group were responsible for the strong adsorption observed with oxidized lipides.

The fact that no correlation could be established between the number of hydroperoxide and carbonyl groups in the unreacted linoleie acid polymers and the extent of complex formation suggested that these groupings were not chemically tied up in the lipideprotein complex reaction. Increasing molecular complexity of the unsaturated fatty acid seemed to enhance the complex formation. O'Connor *et al.* have studied the infrared spectra of several saturated fatty acids and have indicated that the 10.6 micron band obeys Beer's law (16). On this basis it is possible to suggest that the progressive decrease observed in the 10.6 micron band in the infrared spectra of the unreacted linoleic acids could be taken as indicative of increasing polymerization. It will be observed that the hydrolysate obtained from the complexes also did not exhibit the broad band at 10.6 microns in spite of the fact that they gave rise to rather high acid numbers. Therefore, on this basis, the hydrolysates must also be of a highly polymeric nature. Since a strong alkali had been used for the hydrolysis, it is not possible to state conclusively the nature of the molecular complexity of the unsaturated fatty acid in association with the protein.

Since the number of reactive groups in the protein of the complex were almost the same as that in the original protein, it can be stated that the complex formation does not involve a chemical combination between the reactive groups of the lipide and the amino, sulfhydryl, or hydroxyl groups of the protein. It should be pointed out that the carboxyl groups in the protein were not estimated in the method employed in the present study. It has been suggested that the hydroperoxide groups in oxidized fats and fatty acids could react with the carboxyl groups of the protein and form ester linkages (17). A similar reaction does not seem to apply in the case of lipideprotein complexes because no definite relationship could be drawn between the number of hydroperoxide groups in the lipide and the extent of complex formation. However it was difficult to account for the extremely stable nature of the complexes. The strong adsorption obtained with alumina and oxidized lipides seemed to suggest that stable adsorption complexes could be obtained, provided the necessary functional groups were present.

The formation of hydrogen bonds between two strongly electronegative atoms, such as oxygen or fluorine occurring in covalent molecules, is well known. Hydrogen is also known to bridge ionic molecules, such as hydrogen fluoride. Since hydrogen bonding is essentially a weak electrostatic linkage, there does not seem to be any reason to doubt that hydrogen cannot bridge two molecules, one an ionic molecule, such as alumina, and the other a covalent molecule, such as oxidized lipide. In order to express this more quantitatively it can be stated, on the basis of Pauling's scale of eleetronegativities, that the bonding between oxygen and aluminum in the alumina molecule must possess at least 63% of an ionic character as compared with the 39% ionic character of the bond between oxygen and hydrogen in water (18). It is doubtful whether these figures have much significance in the case of ionic linkages however; the order of their magnitude must have some bearing on the electrostatic nature of such linkages. On this scmiquantitative basis a strong hydrogen bonding between the hydrogen of the hydroperoxy or hydroxy group in the oxidized lipide and the oxygen of the alumina can be postulated. This would explain the ease of formation of these adsorption complexes, also the reason why oxidized linoleic acid with a high peroxide number is adsorbed to a greater extent than thermally oxidized corn oil with a low peroxide value.

A similar reasoning could be applied to the case of lipide-protein complexes. The linoleic acid polymer may be considered as being held by means of strong hydrogen bonding to a protein monolayer consisting of approximately parallel peptide chains in an extended *beta* keratin-like configuration. Such a configuration for denatured egg albumin has been proposed by Palmer (19). It is possible that four linoleic acid polymers consisting of three residues each *(i.e.,* a trimer) are adsorbed in the plane of the protein monolayer and also are parallel to the peptide chains in order to account for a larger number of hydrogen bonds. The hydrogen bonding may exist between the hydrogen of the hydroxy and hydroperoxy groups in the oxidized linoleic acid and the oxygen of the earbonyl groups in the protein and also between the oxygen of the earbonyl groups of the lipide and the hydrogen of the amide groups of the protein. Although hydrogen bonds are individually weak (of the order of 5 to 8 Keal/mole), by combining their forces, the cumulative effect of all these forces may be quite large (20). The stability of the urea complexes of longchain, aliphatic molecules has been attributed to the hydrogen bonding between adjacent urea molecules that wind around the long-chain molecule, giving rise to specific crystal structures (21).

On the basis of the above structure for the complex the nonreactivity of lauric acid and unoxidized linoleic acid can be explained as resulting from the lack of hydroperoxy and keto groups in these lipides. It is possible that other forces besides hydrogen bonding are also involved because, on the basis of the above postulate, the linoleie acid polymer containing the largest number of hydroperoxy, hydroxy, and carbonyl groups must complex to the greatest extent with the protein, which however was not the case.

Summary

Complexes have been formed between egg albumin and oxidized linoleic acid. Little or no complex formation could be obtained with unoxidized linoleic acid, oleic acid, or laurie acid. The amount of complexed linoleic acid could not be increased to more than 8.0% in spite of a long reaction time. The number of reactive groups (amino, sulfhydryl, and hydroxyl) in the protein of the complexes and in the original protein were found to be the same. The data seemed to indicate that a covalent linkage did not exist between the reactive groups of the protein and the lipide in complexes of this type. Strongly bound adsorption complexes were formed between alumina and oxidized lipides by a simple mixing technique. A possible structure on the basis of a large number of hydrogen bonds has been proposed for the lipide-protein and the lipide-alumina complexes and has been discussed in detail.

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ABSTRACTS • • • R. A. REINERS, Editor

ABSTRACTORS: Lenore Petschaft Africk, S. S. Chang, Sini'tiro Kawamura, F. A. Kummerow, Joseph Mckoughlin Jr., and Dorothy M. Rathrnann

• Oils and Fats

Function and valuation of fats, oils, and emulsifiers in bakery and food production. S. Young. *3rd Intern. Bread Congr. Hamburg* 1955, 285-92. The activity of distilled monoglyceride in baked cakes would be expected to be proportional to its monoester content or about 2.2 times more active than the reaction mixture. The distilled monoesters in an experimental cake were considerably more active than was predicted. This greater activity was primarily due to the absence of the diester. $(C. A. 51, 15032)$

Stabilization of edible fats by spices, If. A new **antioxidant** fr**om betel leaf.** S. C. Sethi and J. S. Aggarwal(Natl. Chem.
Lab. India, Poona, India). *J. Sci. Ind. Res.(India)* 15**B,** 34–6 (1956). *ttydroxychavicol(4-a]lylpyrocatechol)* isolated from betal leaf has been shown to exert an antioxidant effect on refined peanut oil and refined lard. Ascorbie acid from red chillies was synergistic to their natural antioxidants; eapsaicin and isoeugenol also showed an antioxidant effect on peanut oil. $(C. A. 51, 15031)$

The **role of** lipides in baking. N. Fisher, Margaret L. Ritchie and J. B. M. Coppock(Baking Inds. Res. Sta., Chorleywood, Eng.). *Chem. & Ind.(London)* 1957, 765–6. New data confirm the existence of lipide-protein complexes in flour. Lipide still in combination with protein is extracted with cold carbon tetrachloride from untreated, unbleached 72% extraction flour. The protein moiety varies according to the moisture content of the flour and of the extracting solvent. Acid hydrolysis of the extracted product shows the presence of proline, whereas flour dried and extracted in a desiccator with anhydrous carbon tetrachloride gives no proline reaction. The sterol fraction crystallizes from a hot 97% acetone extract of the acetone insoluble fraction of flour oil on cooling. Sitosterol palmitate is isolated by alcohol crystallization of the product. A countercurrent distribution study of the acetone insoluble fraction

was done with a 24-tube apparatus. Analyses of hydrolyzates show that all fractions contain polypeptide or protein, glycerol, and P and N. $(C. A. 51, 15027)$

The effects of **surface-active agents on baking technology.** E. Maes. *3rd Intern. Bread Congr. Hamburg* 1955, 255-8. Review, 80 references. (C. A. 51, 15023)

Rapid determination of cholesterol in foods. H. R. Marangoni. *Arch. farm. y bioquim Tucumdn* 7, 237-52(1955). Cholesterol is determined colorimetrically by the color produced on heating it with acetic anhydride and sulfuric acid. Methods are given **for** the analysis of ice cream, cookies, butter and other foodstuffs. $(C. A. 51, 15024)$

Replacement of glycerol with xylitol in preparation of **rosin acids esters.** G. A. Fridman. *Gidroliz. i Lesokhim. Prom.* 10 (4), 11-14(1957). Rosin acids were esterified with xylitol under various conditions. As the amount of xylitol was increased from 19 to 28% of the weight of rosin acids, the esterification time was shortened by more than half. The optimum amount of xylitol was found to be 25% ; and the optimum temperature was between 280° and 290°. Above 300° thermal decomposition occurred. The addition of 0.1% of CaO and 0.15% of calcium hydroxide shortened the reaction time from 15 to 10 hours. The reaction rate was accelerated and the product was brighter when the esterificatlon was carried out in a carbon dioxide atmosphere. $(C. A. 51, 15154)$

Sunflower **as oilseeds and fodder** crop in **West Bengal.** H. C. Choudhuri and H. T. Banerjee(State Agr. Res. Inst. Govt. W. Bengal, Calcutta). *Sci. and Culture(Calcutta)* 21(11), 675-7 (1956). Sunflower *(He~iathus annuus)* seeds of the Giant Russian, West Bengal (as a control), Sunrise inbred advance female parent and Sunrise varieties were grown. The semimatured stems and leaves were analyzed for moisture, protein, fat, soluble carbohydrate, fiber and ash. The plants of the Giant Russian variety were best for grain and fodder yields but lower than the plants of the West Bengal variety in oil