TABLE IV
Gas Chromatographic Analysis of Octadecadienoate and of Its Crystal and Filtrate Fractions

Total diene *trans* 44.7% Conjugatable diene 19.7%		Fractions			
		Crystalline (28%) *trans* 52.1% Conjugatable diene 26.6%		Filtrate (72%) *trans* 37.9% Conjugatable diene 14.6%	
Peak	% Wt.	Peak	% Wt.	Peak	% Wt.
A	30.8	A	14.1	A	34.0
B	42.0	B	19.9	B	47.7
C	20.1	C	40.7	C	15.7
D	7.1	D	24.1	D	2.6
....	.....	E	1.2	....	.....

complex relation of *cis,trans* isomers of octadecadienoates to their solubility characteristics. Thus the proportions of *trans* esters change only slightly on fractional crystallization. Isomers corresponding to peaks C and D tend to concentrate in the crystal fraction, and the *trans* content in this fraction also increases somewhat.

Oxidative cleavage of the dienoic acids results in two dibasic acids, one that includes the original carboxyl and the other that is formed from the carbon atoms between the double bonds. The shorter dibasic acids are largely formed from this group between the double bonds. For example, C_6 dibasic acid is formed from 9,15 dienoic acid which results from hydrogenation of the 12 bond. The 50% value for C_9 acids results from the large amount of acids in which the 9 bond is still in the original position.

The triene fraction appears to undergo comparatively little change during hydrogenation. Its iodine value is 250, and it contains about 2% conjugated diene but no conjugated triene. Alkali conjugatable triene comprises 97.3% of the fraction.

Acknowledgments

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The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

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Some Characteristics of the Membranes Protecting Oil Emulsions in Protein Solutions[1]

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Expressed olive juice contains emulsified globules of olive oil. Electron photomicrographs of these globules show a rough membrane protecting them against coalescence. Some features of the membranes, such as the existence of "poles" and deposited microcrystals, are disclosed by the photographs and by electron diffraction patterns. Heavy metals appear to concentrate in the membranes and not in the aqueous medium.

WHEN AN OIL PHASE is dispersed into a water phase, or conversely, the droplets formed tend to coalesce because of surface forces, cohesion, and differences in density. The emulsion is stabilized when these forces are counteracted by other forces preventing the droplets from coming into direct contact with one another. The viscosity of the continuous phase plays a role here, but a more effective prevention is the formation of a barrier of electric charges surrounding the droplets or of a thin film of a third substance with affinity for both phases. Both may act simultaneously.

Natural emulsions of the "oil-in-water" type are often stabilized by lipoproteins, which form a very thin layer surrounding the droplets. This discovery was made by Ascherson who proposed the name "haptogen membranes" for these films in 1840. Milk is a typical example of this kind of emulsion and the one most extensively studied to date. Palmer and coworkers devoted 20 years to the study of the membranes protecting the fat droplets in milk, and the techniques developed by him for the isolation of these membranes have been used by many other investigators to study milk and other natural emulsions. A recent book on this subject (1) constitutes a very useful up-to-date study with many references.

Our interest in these studies was aroused because an oil-in-water emulsion also forms when olives are ground in the first step for the extraction of their oil. In this emulsion the olive oil droplets are protected by lipoproteic membranes formed at the expense of the substances dissolved in the juice of the olives. The extraction consists of two steps: separation of the emulsion from the solids present in the ground paste, and settling or centrifugation to free

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the oil from the water phase. Difficulties arise in the settling because the milling and beating action decreases the density differential between water and oil phases. Thus, from the density of the membranes and their mean thickness, calculations show that droplets less than 20 microns in diameter have the same specific gravity as the liquid medium. They cannot be separated by centrifugation alone. It is estimated that every year in Spain about 10 million pounds of oil are lost in the emulsion of oil present in the waste liquid from the olive-oil mills. The presence of the membranes interferes in the separation by making the flow of the oil droplets through the solid particles of the paste more difficult. Consequently we are devoting much attention to the study of these emulsions.

The more stable emulsions are formed during the milling and extraction. The oil occurs in the cells of the olive fruit in tiny droplets, about 2 microns in diameter. These droplets however easily join together to make greater droplets, and the coalescence can be observed under the optical microscope. The stable emulsions begin to form only after the tissue structure has been destroyed by crushing and grinding and the juice of the fruit is liberated. Thus the milling and beating processes are a compromise between the need for destroying the tissue to free the oil and the inconvenience of emulsion formation. By the beating action, most of the oil is segregated into large globules or continuous layers easy to extract, but the rest of it forms an emulsion. If the oil globules in this emulsion have a diameter less than about 50 microns, they remain unextracted in the solids of the paste or are drained out with the waste liquids. Only a minor part can be recovered from the liquid wastes by centrifugation or decantation.

Emulsions similar to those formed in the oil mills can be prepared in the laboratory by stirring a mixture of olive oil with the filtered water-phase from oil extraction (called in Spain *alpechines*). The general features of these emulsions and the techniques followed for the isolation and analysis of the membranes are described in previous papers (2). In a communication presented at the Third Congress of the International Society for Fatscience (3) we reported our studies on methods of destroying membranes and of inhibiting their formation by the addition of small quantities of alkyl-aryl-sulfonates.

In this paper we describe more recent studies on the structure of the membranes and on their ability to adsorb atoms or ions of heavy metals selectively.

Electron Microscope Studies on Structure of the Membranes

Emulsions of olive oil in the juice of the olives have proved to be very good subjects for study by electron microscopy. The only necessary pretreatment is dilution (about 1:1,000) of the samples. No shadowing was employed in most of the photographs obtained, and no replica technique was needed.

A series of about 600 photographs was taken by one of our collaborators in the laboratories of the Centre National de la Recherche Scientifique near Paris. We have selected some of these and some of another series taken in the Instituto de Optica and in the Instituto Agronómico, both in Madrid. The first three figures support, by photographic evidence, information already established by other techniques.

Figure 1 (negative) shows a droplet 2.2 microns in diameter with shadowing with gold and palladium. The membrane is clearly shown at the edges of the droplet and in the rugosity or wrinkling of the surface. Figure 2 shows a droplet of approximately the same size without any shadowing. Some detail on the

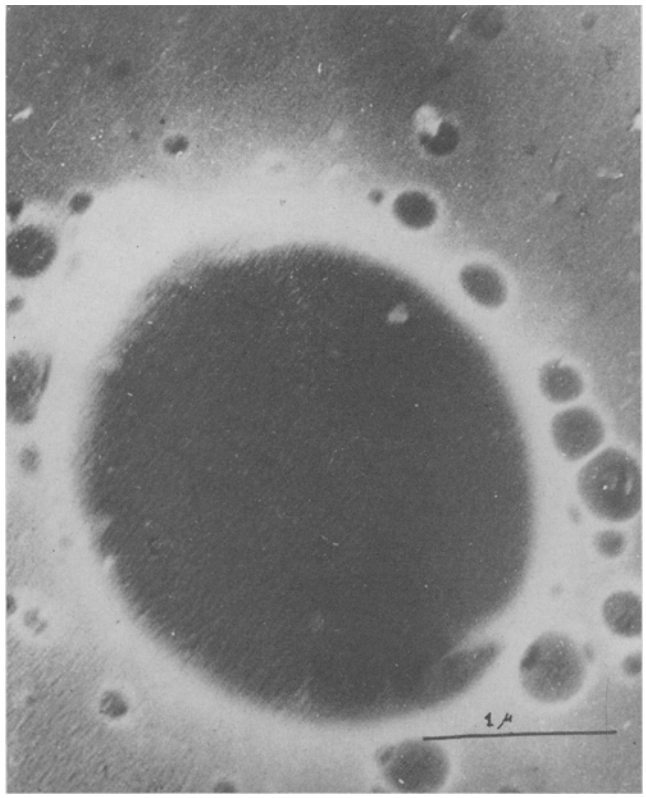

FIG. 1. (Negative). Globule of olive oil 2.2 microns in diameter. Shadowing with gold and palladium.

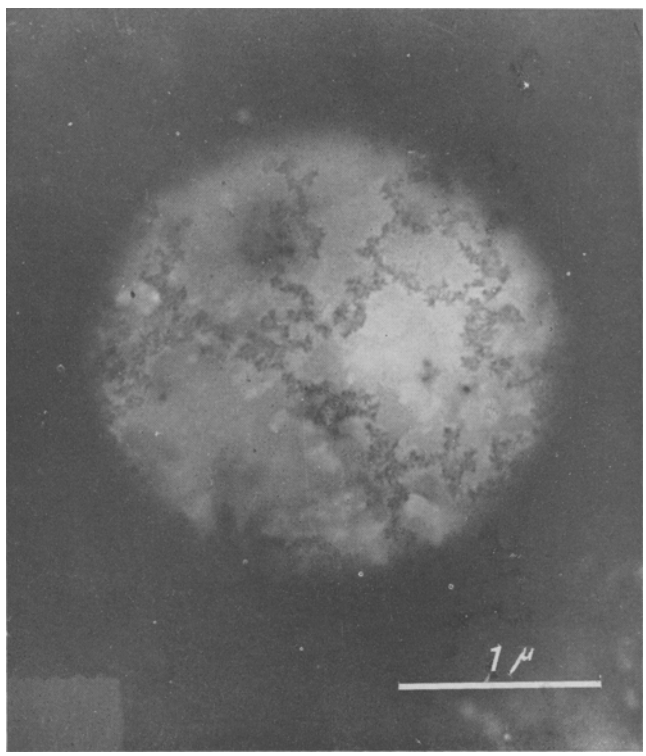

FIG. 2. Globule showing microcrystals on surface. No shadowing.

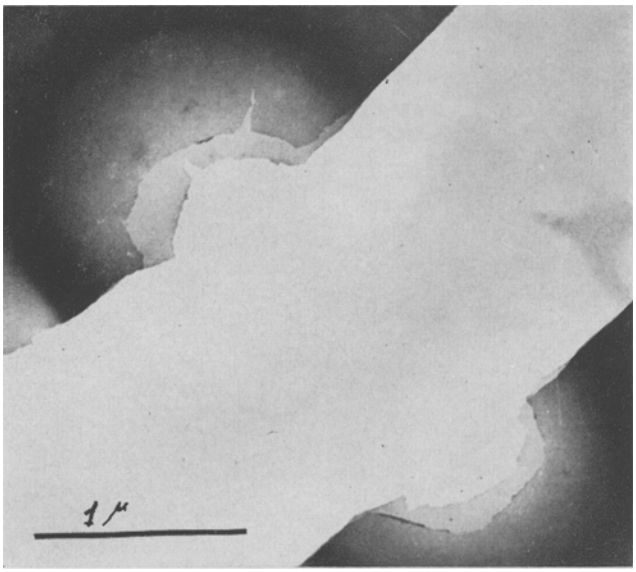

FIG. 3. Rupture of globule showing layers.

surface is readily discernible, and microcrystals of metallic salts are believed to be precipitated on the surface.

Figure 3 shows the splitting of an oil globule by a beam of electrons. The membrane looks like an egg shell and appears to have the characteristics of a solid. Two layers may be seen in the shell. This picture was obtained by increasing the strength of the electron beam directed through a droplet supported on a film of cellophane. Although it was known that such ruptures were frequent, it required many photographs to catch a rupture across the middle of a droplet.

The electron photomicrographs show other characteristics of these droplets and their membranes that we have found difficult to interpret. One group of these characteristics is associated with a singular spot or pole on the membrane and the other group with clusters formed when emulsions were formed by exposure to ultrasonic waves. The "pole" occurs in many of the droplets where the membrane is thicker. It is often found on the upper side of the droplets as shown in Figure 4 (arrow). The pole is much easier to observe when it is on the edge or in profile position (Figure 5). The frequency of the observation of these poles approached 50%, suggesting that every membrane contained a pole.

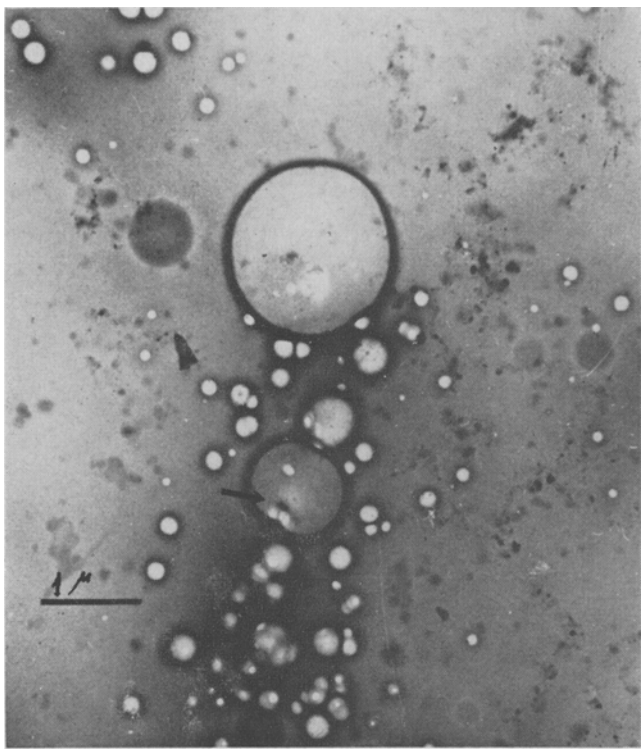

FIG. 4. Fat globules with pole. Arrow points to pole.

It is difficult to explain the reason for the existence of these poles. They could be holes in the membrane through which the oil is beginning to leave and to spread out the residue of the membrane. However the presence of a hole does not explain the greater apparent thickness of the membrane adjacent to this pole or the gradual decrease in the apparent thickness away from the hole. Such thicknesses are common to certain other natural sphincters. It is also difficult to explain why the breakdown of the membrane should occur in one single spot each time.

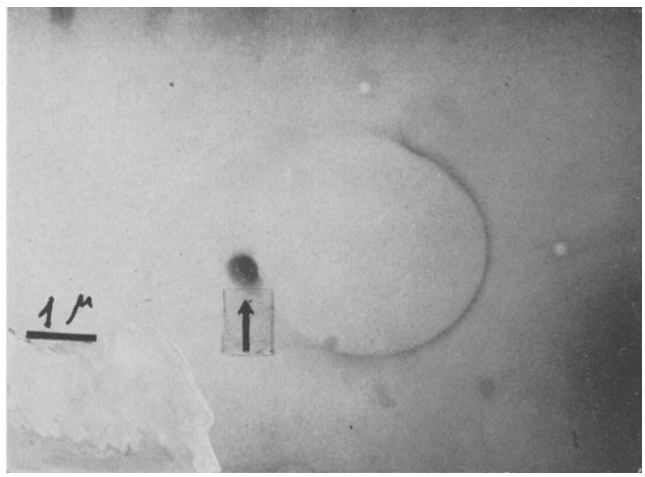

FIG. 5. Fat globule with pole. Arrow points to pole.

Another curious phenomenon is the subdivision of droplets observed when emulsions are prepared by means of ultrasonics. Simple mechanical stirring results in emulsions wherein the droplets are isolated and are fairly constant in size (1 to 5 microns). Droplets greater than the field of the microscope were seldom observed. Interfacial tensions, viscosity, and other physical factors effected by the emulsifying, thickening, stabilizing, and other factors present are probably responsible for this. When ultrasonics are employed, the droplets tend to appear as clusters, with the forms of these clusters suggesting to us that they were formed from greater drops by subdivision. Nevertheless it is difficult to explain how the membrane walls in the cluster shown in Figure 6 developed from the previous membrane. In Figure 7 the emulsion was treated with a small quantity of a weak solution of trichloroacetic acid to give thinner membranes and to permit better observation of the inner subdivisions.

In Figure 8 an odd formation is shown that was apparently formed by several droplets coming to-

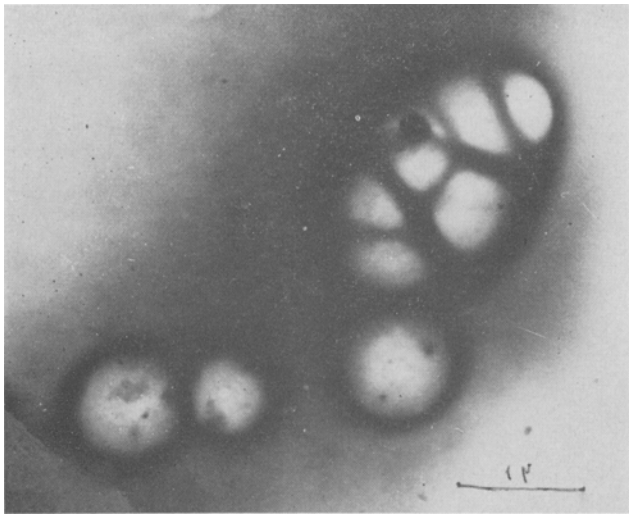

FIG. 6. Typical cluster of fat globules from emulsion prepared with ultrasonic waves.

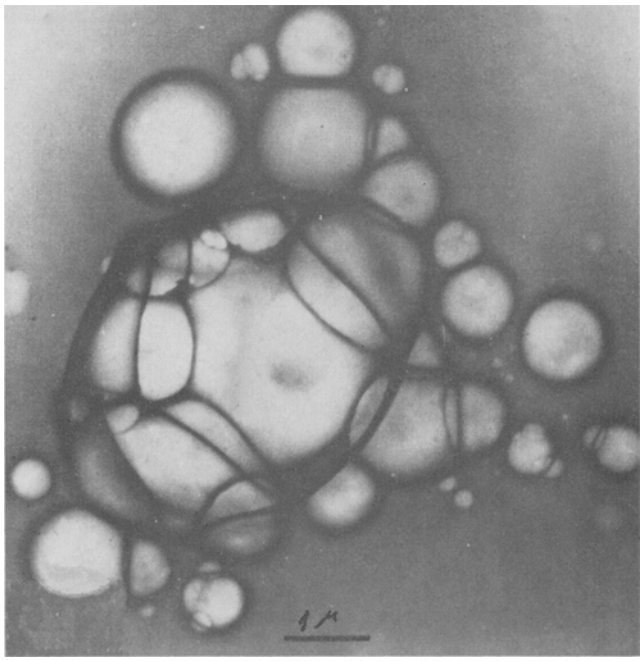

FIG. 7. Cluster from emulsion treated with trichloroacetic acid.

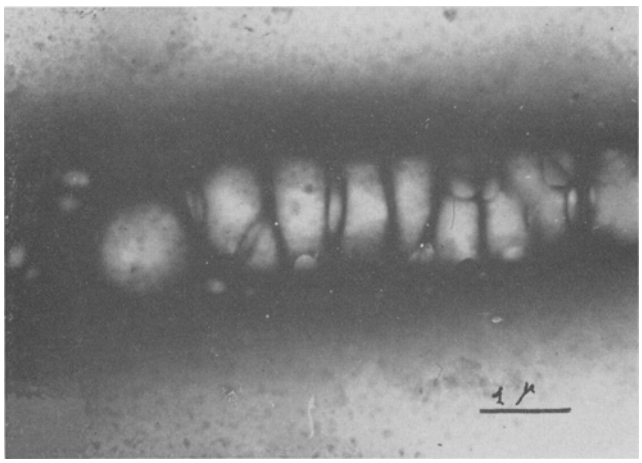

FIG. 8. Odd cluster formation.

gether. The greater thickness of the external walls of the tube may be noted.

Unfortunately we have not, up to date, been able to obtain electron photomicrographs of the droplets as they are in the undamaged olive fruit. This would, of course, necessitate more sophisticated techniques, such as special microtome, fixation, etc., which were not available in the laboratories where we worked on the subject. The membranes of the droplets in the fruit must be very thin for these droplets easily join together whenever they come into direct contact. But a better knowledge of the structure surrounding the droplets in the fruit would be very helpful for interpreting some details of the photographs taken from emulsions in the fruit juice because undoubtedly some of these details must be remainders of the vegetable structure.

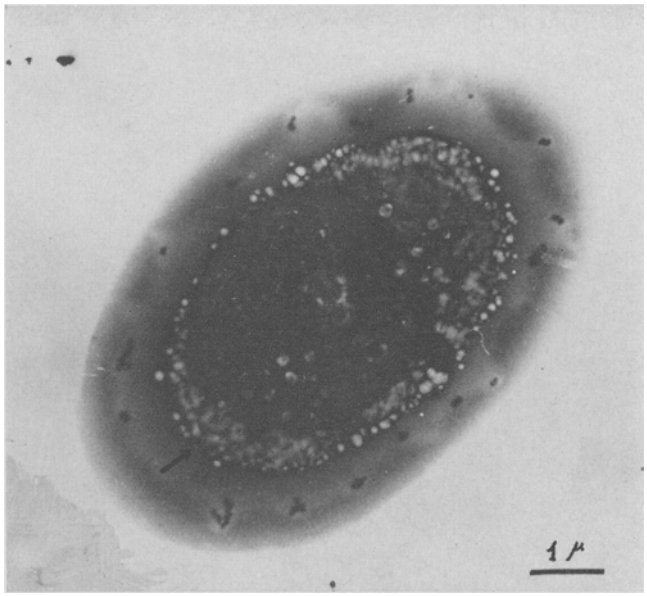

FIG. 9. Globule found in emulsion from immature olives.

Figure 9 shows a formation that arises only in the juice of olives picked in the very early stages (four months before maturity). Normal droplets like those shown in Figures 1 to 4 were present also. Note that the droplet is surrounded by a sort of conglomerate with a great stain with neat edges including the whole formation. This formation might be better understood if we had photomicrographs of the undamaged fruit at different stages of growth.

Electron Diffraction at the Membranes

It is commonly admitted that the membranes are formed, at least in the inner layers, of radially-oriented molecules. The following scheme has been proposed for the structure of the membranes of the fat globule in milk. As shown in Figure 10, the innermost layer is formed of high-melting triglycerides radially oriented. Next there is a layer of phospholipids with its fatty acid chains parallel to the triglycerides, with the polar groups oriented outward and confronting the hydrophilic side chains of the proteins. The long polypeptide axes of the protein are stretched parallel to the globule surface. This structure should give a diffraction pattern when the electron beam is directed for diffraction at the membrane. Figure 11 shows

such a pattern, but it probably corresponds to the pattern of metallic salts adsorbed in the membrane. However with a droplet that appeared to be compressed against a solid particle we were able to obtain

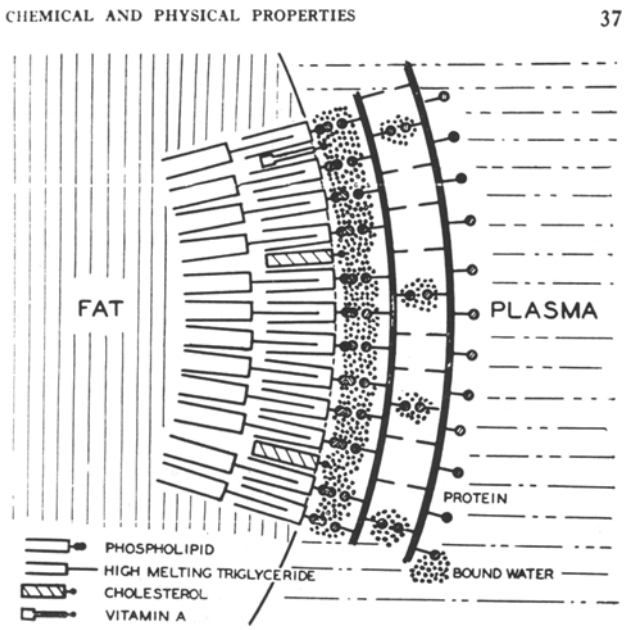

FIG. 10. Graphic representation of composition of interface.

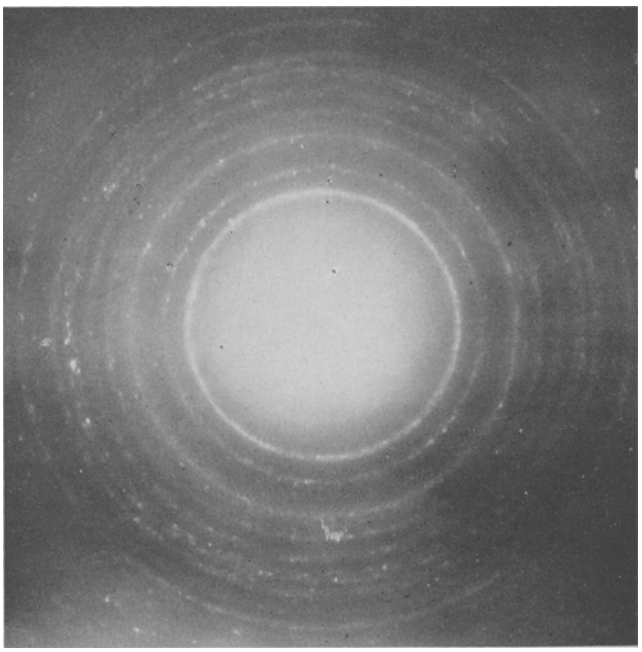

FIG. 11. Diffraction pattern of fat globule, using electron microscope.

an orthorombic pattern (Figure 12). It is the opinion of collaborators in Madrid that this pattern was indeed caused by oriented molecules in the membrane.

Observations in the optical microscope with polarized light furnish additional evidence that a semicrystalline phase is present in the inner layers of the membranes. Similar views are held by King (1), who observed a birefringent layer in milk droplets when compressed.

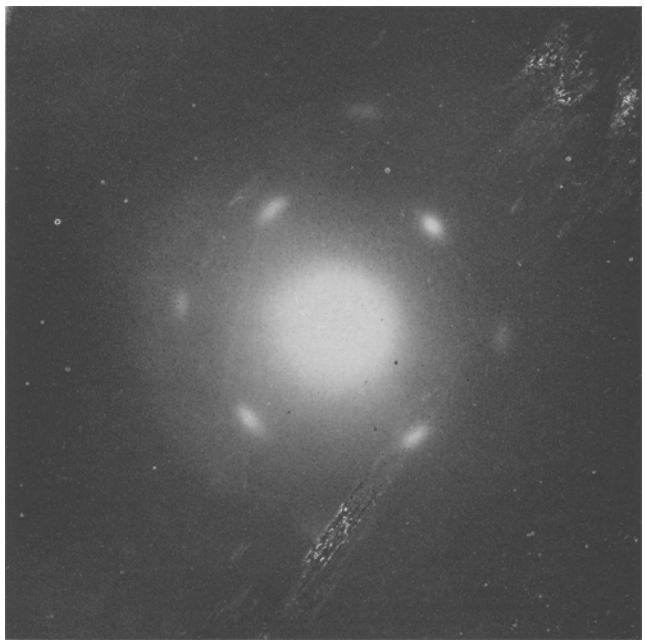

FIG. 12. Diffraction pattern of compressed globule.

Heavy Metals in the Membranes

The opacity of the membranes to the electrons probably results from the presence of traces of heavy metals adsorbed from the main solution. Analyses of isolated membranes for copper, iron, and other heavier metals showed that the membranes contained many times greater amounts than the solution. Light metals, such as potassium, are present in fairly high concentrations in the solution but are not concentrated in the membranes. The table shows data on concentration of a number of metals including potassium in membranes and juice. In Figure 13 the concentration of uranium in the membranes and filtered juice was determined after the addition of small quantities of uranyl nitrate to the filtered olive juice, followed by emulsification of the mixture. Membranes were then isolated, calcinated, and ashes analyzed for uranium.

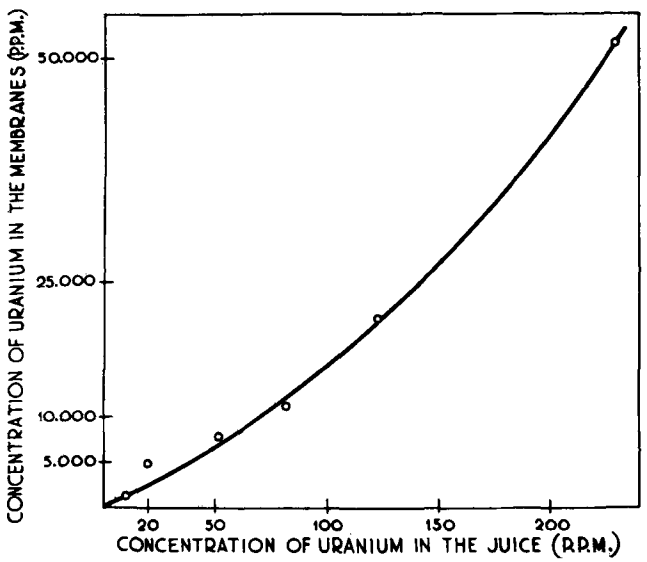

FIG. 13. Variation of uranium concentration in membranes from that in olive juice.

The comparatively large amounts of uranium and the form of the curve suggest that chemical combination and perhaps solvation of metallic ions contribute to the high uranium content. More work will be needed before definite conclusions can be drawn.

TABLE I

Metallic Content of Emulsion Fractions

Metals	Oil	Vegetation water	Membranes
Iron, p.p.m.	1.9	54	3890
Copper, p.p.m.	0.02	8.6	1300
Zinc, p.p.m.	0.1	19.9	3915
Manganese, p.p.m.	0.015	3.3	53.4
Potassium, p.p.m.	0.00	8000	1950
Ash, %	0.014	1.97	9.2

Acknowledgment

The authors want to express their gratitude to Professor Trillat, head of the laboratory for electron microscopy of the Centre National de la Recherche Scientifique of France, where most of the photographs of the droplets were made. Thanks are also due to the Spanish Fundación March for the scholarship accorder to Márquez Delgado.

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The Temperature Dependence of Micellar Solubilization

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The temperature dependence of micellar solubilization was determined in the 180°–140°F. temperature range by using a dye solubilization technique with built and unbuilt solutions of three high-cloud point, commercial surfactants, one anionic of the alkylaryl sulphonate type and two types of nonionic agents. It was found that the logarithm of solubilization in both built and unbuilt solutions was directly proportional to temperature (solubilization was an exponential function of temperature) and that the log solubilization-temperature slopes of the built and unbuilt solutions of each surfactant were approximately parallel.

MICELLAR SOLUBILIZATION is considered by some investigators to be one of the first actions of the detergency mechanism (1). It is therefore important to know how solubilization varies with temperature under field conditions. While hard surface, aqueous cleaning is usually carried out at elevated temperatures, most of the literature data on solubilization are in the low 77°–122°F. (25°–50°C.) range (2,3,4,5,6,7). These data are not complete enough to permit the derivation of solubilization-temperature functions. But it is possible to postulate that in this range solubilizing activity increases with increasing temperature and the temperature coefficient of solubilization varies with the surfactant (5).

This paper deals with the temperature dependence of the micellar solubilizing power of three important types of surfactants in the 180°–140°F. temperature range. Solutions of all three surfactants, two nonionic (including a polyoxyethylated alkyl phenol) and one anionic, possessed high-cloud points and were clear in the temperature range studied. A dye solubilization technique was used with built and unbuilt surfactant solutions. Surfactant and builder concentrations were those obtaining in practical detergency.

Materials

Surfactants. Those used in this investigation were 100% active, commercial products. Two were nonionic agents, polyoxyethylene sorbitan monolaurate (PSML) with a molecular weight of 1226 (according to the manufacturer) and pentadecaethylene glycol nonyl phenyl ether (PGNPE), molecular weight 880. The third surfactant was an anionic agent of the alkyl arylsulphonate type, sodium dodecyl benzene sulphonate (SDBS), molecular weight 346.

Dye. Used in the as-received condition, this was Orange OT, 1-O-tolylazo-2-naphthol.

Builders. These were A.C.S. Na_2SO_4 and technical grade $Na_5P_3O_{10}$.

Experimental

The experimental technique was that used in a previous study of micellar solubilization (8), with the following changes primarily because of the higher temperatures.

a) Preheating time in the water bath prior to addition of dye was increased to 35 min. The interaction period remained 25 min.

b) The interaction test tubes were given a repetition of the initial mixing at 8 and 16 min. after addition of dye to surfactant solution.

c) Filtration was into a receiver placed in the thermostated water-bath.

d) Aliquots of the filtrate were diluted with 1:1, acetone-water mixture prior to determination of optical density at a wavelength of 425 millimicrons. The dilution was made by pouring slightly less than 5 ml. of the hot filtrate into an ice-cooled, 25-ml. glass-stoppered type of graduated cylinder containing 20 ml. of 1:1, acetone-water mixture measured at room temperature. The cylinder was stoppered, the contents were mixed and brought to room temperature, and the volume was noted. The size of the aliquot was given by the difference in volume.

Results and Discussion

Table I gives the solubilization of Orange OT (each value the average of at least two determinations) at temperatures of 180°, 160°, and 140°F. in solutions of surfactants PGNPE, PSML, and SDBS in distilled water and in 0.025M and 0.05M Na_2SO_4 and $Na_5P_3O_{10}$, respectively. For each temperature, solubilizations are given for three concentrations of each surfactant. Some of the data are plotted in Figure 1. The data and graphs indicate that in the 180°–140°F. range the logarithm of solubilization is directly pro-