Summary

It has been shown that the oil of *Laemonema Morosum* Matsubara, a deep sea fish, contains a large amount (31–34%) of unsaponifiable matter. The main component (*ca.* 50%) of which is 11-docosen-1-ol, has an m.p. 31.7–32.3°C. This alcohol has not been previously reported in the literature. From this alcohol *trans*-11-docosen-1-ol having m.p. 52.4–52.8° C. was prepared. The infrared spectra of these two alcohols are given.

Acknowledgment

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Studies on the Oxygen Uptake of Fat Emulsions Used in Intravenous Alimentation[1]

ROBERT P. GEYER, IRVING SASLAW, and FREDRICK J. STARE, Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts

THE PARTICLES of fat in an emulsion which is suitable for intravenous nutritional purposes (1, 2, 3, 4) must be extremely small to avoid undesirable physiological reactions and to insure maximum stability. In such emulsions most of the particles of fat are well below 1 micron in diameter, resulting in an enormous surface area of lipids which might favor hydrolytic and oxidative reactions. Preliminary experiments, however, in which a coconut oil emulsion (1) was used, disclosed almost complete stability to both types of degradation reactions under normal conditions of storage.[2] To gain a better understanding of the factors involved in the oxidation of the highly dispersed lipid in such emulsions, a number of experiments were subsequently done in which the uptake of molecular oxygen by various emulsions and their individual components was studied manometrically. As pointed out by Holman (5), relatively little work has been done on the oxidation of emulsions of fat.

The problems investigated were a) the relative rates of oxidation of a typical emulsion and its components, b) the influence of the type of oil on the uptake of oxygen by emulsions, c) the influence of the type and concentration of stabilizer on the uptake of oxygen by both emulsions and the stabilizers themselves, d) the influence of particle size of the oil droplets on the oxygen uptake of emulsions, and e) the influence of temperature on the oxidation rate of emulsions and stabilizers. Although the conditions used for these measurements were not those dealt with in the actual use of the emulsions, they allowed comparisons to be made between various preparations in a relatively short period of time. Furthermore data so obtained should prove useful as a complement to results found when the more usual but more time-consuming procedures are used. The usefulness of the Warburg manometric method in studies on the oxygen uptake of oils has been shown (6, 7, 8).

Experimental

Preparation of Emulsions. The preparations used in the present studies were made by means of high pressure homogenization. The homogenizer was preheated to approximately 65°C. by means of hot water, rinsed with distilled water, and then flushed with nitrogen. The emulsion ingredients were added to the required amount of warm 5% dextrose solution, and the entire mixture was premixed in a blender for 5 min. The blender jar was provided with a thermometer and an inlet tube for nitrogen. The preparation was then transferred to the homogenizer[3] and emulsified at 3,000 lbs. p.s.i. until the diameter of the particles was well below 1 micron as determined by visual phase microscopy. Samples removed before the particles were this small are so designated under the proper experiment. In all cases the temperature was kept below 85°C., and a nitrogen atmosphere was maintained over the liquid in the reservoir. The finished emulsions were sealed in glass containers after flooding with nitrogen and autoclaved for 15 min. at 15 lbs. p.s.i. The sterile preparations were stored in the dark at room temperature and were used within two days from the time of preparation.

Method of Studying the Oxygen Uptake. The oxygen uptake was measured by conventional technique (9), using a Precision circular, twenty-place Warburg apparatus at 38°C. The shaking speed was 90 strokes per minute with an amplitude of 3 cm. Flasks with a capacity of 15 ml. were used, and the total liquid volume in the flask was 3 ml. Gassing was done with 100% oxygen for 10 min. and was followed by a 5-min. equilibration period. Resetting of the manometers was accomplished when necessary by opening the stopcock to air.

Oxygen Uptake Experiments. A large number of

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[2] Unpublished data obtained in this laboratory.

[3] Model 124 E, Manton Gaulin Manufacturing Company Inc., Everett, Mass.

experiments were done in which the oxygen uptakes of various emulsions, components of emulsions, and supplementary compounds were determined. In all cases 5% dextrose solution made up the aqueous phase. Some of the most pertinent experiments follow:

1. The oxidation of a clinical type of emulsion (1) and its individual components was studied, using the following substrates:

a) emulsion containing 15% coconut oil,[4] 0.5% soybean phosphatide,[5] and 1% Demal-14[6];

b) emulsified 0.5% soybean phosphatide;

c) emulsified 1% Demal-14; and

d) emulsified 0.5% phosphatide and 1% Demal-14.

The rate of oxygen uptake by these preparations was followed for 386 min., and the results are presented graphically in Figure 1.

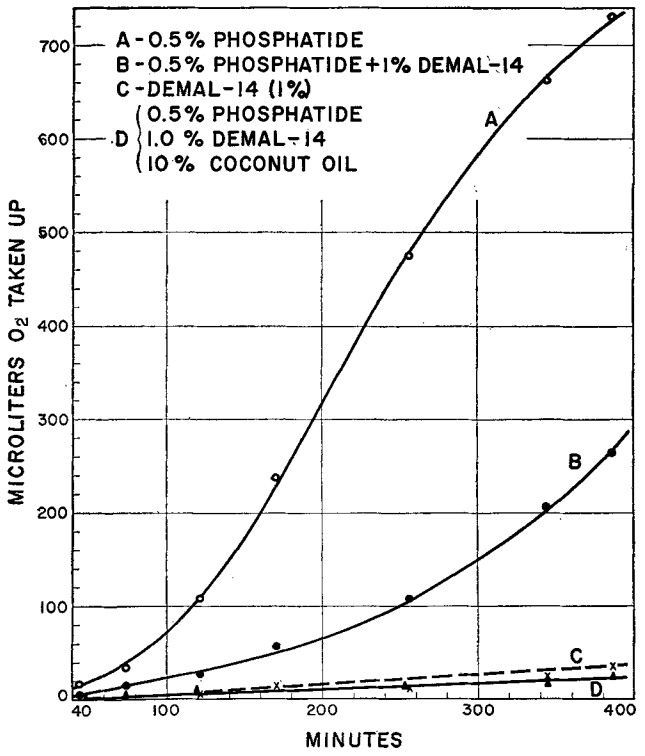

FIG. 1. Oxidation of phosphatide alone and when emulsified with other materials.
An O_2 gas phase and temperature of 38°C. were used.

2. The oxidation of emulsions made with different oils but with the same stabilizing system was studied to determine whether or not the chemical characteristics of the oil modified the rate of oxygen uptake. The emulsions used had the composition of that given above under 1. a) except that the oil used was one of the following: coconut oil, hydrogenated coconut oil,[7] corn oil,[8] sesame oil,[9] or linseed oil. The results of these studies are given in Table I and are expressed in terms of the total oxygen uptake per flask. Results

[4] Cobee brand 76, E. F. Drew and Company, Inc., New York, N. Y.
[5] Triply reprecipitated phosphatides prepared from crude lecithin, grade N, The Glidden Company, Chicago, Ill.
[6] Demal-14, a polyglycerol ester of oleic acid. Emcol SPN, a polyglycerol ester of stearic acid. The Emulsol Corporation, Chicago, Ill.
[7] E. F. Drew and Company Inc., New York, N. Y.
[8] Specially refined corn oil, Corn Products Refining Company, Argo, Ill.
[9] Durkee Famous Foods Company, New York, N. Y.

TABLE I

Oxygen Uptake of Phosphatide-Demal Stabilized Emulsions Containing Various Oils[a]

Oil (15%)	Phos. (0.5%)	Demal (1.0%)	Total Microliters O_2 Taken Up (Time in minutes) 40	69	120	177	246	289	350	391
Linseed	+	+	4.0	6.0	18	32	49	59	76	96
Sesame	+	+	0	0	0	7.0	15	24	36	43
Coconut	+	+	0	3.0	4.1	8.2	10	16	23	29
Hydrogenated Coconut	+	+	1.9	2.1	3.0	....	10	11	14	17
Corn	+	+	2.1	2.1	....	4.0	4.0	5.0	10	15
.....	+	+	2.0	8.1	24	52	98	142	214	276
.....	+	....	14	34	108	252	420	528	674	728

[a] All emulsions were made to volume with 5% dextrose solution.

of the oxygen uptake of the emulsified phosphatide and Demal-14 are also included.

3. The following studies were also undertaken:

a) the effect of varying the stabilizer system with or without changing the kind or concentration of the oil;

b) the effect of varying the stabilizer system and changing the kind of oil; and

c) the effect of varying the stabilizer system in oil-free preparations.

Preparations used and the results of some of these studies are given in Table II in terms of the oxygen uptake per flask per 334 min.

TABLE II

Effect of Various Materials on the Oxygen Uptake of Phosphatide

Phosphatide 0.5%	Demal-14 1.0%	Cerebrosides 1.0%	Emcol SPN[a] 1.0%	Gelatin 5.0%	Oil 15.0%	Oxygen Uptake Microliters O_2/334 min.
+						640
	+					29
		+				0
				+		3
+	+					194
+		+				51
+			+			138
+				+		93[b]
+	+				Coconut	19
+		+			Coconut	43
+			+		Coconut	31
				+	Coconut	44
+	+				Corn	10
				+	Corn	1173
+	+				Sesame	32
+			+		Sesame	1
+	+				Linseed	68
+			+		Linseed	76
				+	Linseed	4429

[a] See footnote 4.
[b] This was a poor emulsion because of the incompatibility of the gelatin and phosphatide.

4. The influence of particle size on the oxygen uptake of several types of emulsions was studied. Unfortunately no suitable method is available to prepare emulsions in which the particle sizes are within certain narrow, well-defined limits. Therefore this phase of the present study was investigated in a relative way. Emulsions were made in the usual way, but aliquots were removed at two different intervals during the homogenization and again at the end in order to obtain preparations in which microscopic examination revealed a definite decrease in particle size as the time of homogenization was increased. The emulsions used, pertinent data, and some of the results obtained are given in Table III.

5. The effect of temperature on the oxygen uptake of emulsions and their ingredients was studied by

TABLE III

Effect of the Degree of Emulsification on Oxygen Uptake of Phosphatide and Gelatin Stabilized Emulsions

Oil (15%)	Phosphatide %	Demal-14 %	Gelatin %	Microliters O_2 taken up in 334 min.		
				Poor[a] Emulsion	Fair[b] Emulsion	Good[c] Emulsion
Linseed	....	....	5.0	49	494	4429
Corn	....	....	5.0	10	....	1173
Coconut	....	....	5.0	29	42	44
Coconut	1.5	....	....	....	....	135
Linseed	1.5	....	....	....	....	86
Coconut	0.5	1.0	....	24	20	15
Corn	0.5	1.0	....	16	9	6

[a] Poor—most particles of oil between 3.0 μ – 7.0 μ.
[b] Fair—most particles of oil between 1.0 μ – 3.0 μ.
[c] Good—most particles of oil between 0.5 μ – 1.0 μ.

performing the experiments at 60°C. instead of at 38°. Table IV contains the preparations used, pertinent data, and the results of these experiments.

TABLE IV

Effect of Bath Temperature on the Oxygen Uptake of Various Preparations

Components	Concentration %	Microliters O_2 taken up in 323 min.	
		38°C.	60°C.
DE-3	0.5	614	1844
Demal	1.0	21	56
Gelatin	5.0	3	15
Coconut Oil DE-3	15.0 1.5	126	2599
Coconut Oil DE-3	25.0 3.0	431	3171
Coconut Oil Demal DE-3	15.0 1.0 0.5	18	650
Coconut Oil Gelatin	15.0 5.0	44	200

Results and Discussion

From the data presented in Table I and Figure 1 it can be seen that the rate of uptake of oxygen by highly dispersed fat in an aqueous medium does not equal the sum of the rates of uptake of its component parts. Thus, whereas the emulsion of phosphatide alone took up 72.8 μl. of oxygen, the finished emulsion took up only 2.9 μl. in the same length of time at 38°C. It is apparent that, in such an emulsion, the phosphatide is responsible for the major part of the oxygen uptake and that other materials present in the preparation decrease the rate of O_2 uptake. Even the Demal-14 decreased the oxidation rate of the phosphatide. Replacing the coconut oil with the hydrogenated oil did not alter the oxygen uptake. These data also help to explain the fact that the type of emulsion used here has been found to develop no chemically significant peroxide value even on long standing in contact with air. It must however be emphasized that oxidation may occur which is not significant chemically from a quantitative standpoint but is significant from a physiological standpoint.

That the results obtained with the clinical-type emulsion are not dependent upon the degree of unsaturation of the oil used is well shown by the data in Table I. Emulsions which contained coconut oil, hydrogenated coconut oil, corn oil, sesame oil, and linseed oil all showed the foregoing phenomenon. Even the highly unsaturated linseed oil took up only 9.6 μl. of oxygen in 391 min.

The kind of oil used had very little influence on the oxidation of cerebroside or phosphatide-stabilized emulsions, but, as shown by the data in Table II, it had a marked effect in the case of gelatin-stabilized emulsions. Cerebrosides alone or in combination with Demal or phosphatide took up little oxygen; likewise emulsions of these materials containing various oils did not take up much oxygen. On the other hand, when gelatin was used as the stabilizing system, the oxygen uptake of all oils was increased. In the case of the more unsaturated oils this increase was very great, allowing the oxidation to proceed at an autocatalytic rate. The gelatin itself took up an insignificant amount of oxygen.

The data given in Table III concerning the influence of particle size of the fat droplets on the rate of oxygen uptake show that this influence is dependent upon the kind of stabilizer used. When phosphatide was used as the stabilizing system, the most highly emulsified preparation took up the least amount of oxygen. In contrast, the more highly emulsified the gelatin stabilized preparation was, the more oxygen was consumed. The latter finding is probably a reflection of the increased surface area resulting from the more extensive homogenization.

From the data given in Table IV it can be seen that the uptake of oxygen was greatly increased by raising the temperature from 38 to 60°C. Although most of the preparations tested showed this trend, the differences between the various preparations at one temperature are comparable to those obtained at the other.

The results reported in this paper demonstrate that emulsification of itself need not cause great instability of oils toward oxidation and emphasize the importance of the type of stabilizing system used. It should be pointed out that those systems which were most stable to oxidation were composed of the oil and stabilizers which were at least partially soluble in one another, thus allowing for the formation of a layer or film of the stabilizer at the interface between the water and the oil by virtue of both fat-soluble and polar groups in the same molecule. It would appear possible that, in such a manner, protection could be afforded to the unsaturated fatty acid moieties of both stabilizer and oil since these portions of the molecules would be, instead, surrounded with a film of more polar groupings. It is possible that such an arrangement could function in at least three ways: a) retard the rate of diffusion of oxygen to the reactive sites in the fatty acid moieties; b) keep at least the phosphatide unsaturated fatty acid moieties from enjoying optimum spatial arrangements for a chain reaction type of oxidation; and c) furnish an outer network of groupings, such as phosphate, to scavenge metallic ions which may otherwise act to catalyze the oxidation. The latter has been suggested by others (10) to explain the antioxidant effect of phosphatides. This type of alignment would be unlikely in the case of the gelatin stabilized emulsions. In such emulsions the gelatin would be entirely in the water phase as a true hydrophilic colloid. The fat particles would be held apart by a gelatin-water barrier and not have a film or layer around them, and the net effect would be to facilitate greatly the uptake of oxygen by the oil. Therefore the finer the

particles of fat, the greater the surface area afforded for contact with oxygen.

Further studies are in progress to gain a better insight into the reasons for the differences between the oxidation rates of the various emulsions, also to evaluate the influence of pro- and antioxidants on the rate of oxidation.

Summary

The rate of oxidation of various emulsions designed for intravenous use was studied manometrically. Soybean phosphatide dispersions rapidly absorbed oxygen at 38°C. whereas when used in emulsions containing fat with or without additional stabilizing agents, little oxygen was absorbed even when such unsaturated oils as linseed were used. On the other hand, emulsions of oils stabilized with gelatin took up oxygen rapidly, and the finer the size of the fat particles, the more rapidly was oxygen taken up. At 60°C. all rates of oxidation were increased, but the differences between preparations remained. The results are discussed.

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Effect of Inert Atmosphere in the Determination of Free Fatty Acid or Free Caustic Alkali and Unsaponified Material in Soap

E. W. BLANK and E. EMENDORFER, Colgate-Palmolive Company, Jersey City, New Jersey

IT HAS long been recommended in the determination of the free caustic alkali content of soap and soap products that exposure of the alcohol solution of the sample to atmospheric carbon dioxide should be avoided (2, 4, 5, 6). The precaution stems from the observation that alcohol neutralized to a phenolphthalein endpoint (pink) decolorizes almost instantly when shaken or filtered in contact with the atmosphere due to the absorption of carbon dioxide. The absorption of carbon dioxide by alcohol is appreciable, being approximately 2.5 times as great in alcohol as in water under comparable conditions of temperature and pressure (3). Based on this characteristic of neutral alcohol, low results are to be anticipated in the determination of free caustic alkali unless suitable precautions are taken to work in an inert atmosphere. This is not a simple operation and is usually disregarded save for the use of watch glasses to cover beakers and funnels. No estimate of the magnitude of error involved is to be found in the literature.

During the course of investigational work in this laboratory an apparatus of the dimensions and form shown in Figure I was designed. It was subsequently conceived that this apparatus could be adapted to the determination of both free acid or free caustic alkali and unsaponified material in an inert atmosphere.

Experimental

The apparatus is employed in the following manner. Accurately weigh about 5 g. of the soap sample into the 250-ml. double-necked flask. Pass a slow stream of nitrogen through the flask *via* the small side neck. Add 100 ml. of hot, neutralized alcohol (95% strength or better). Attach the sintered glass filtering unit and flask and, while continuing the flow of nitrogen, place the apparatus on a hot plate. Swirl the contents of the flask occasionally to hasten solution of the sample.

When the sample is in solution, except for alcohol-insoluble salts, remove the flask from the hot plate. Slowly rotate the apparatus to an inverted position so that the alcohol solution in the lower flask filters through the sintered glass filter into the saponification flask without coming in contact with the nitrogen

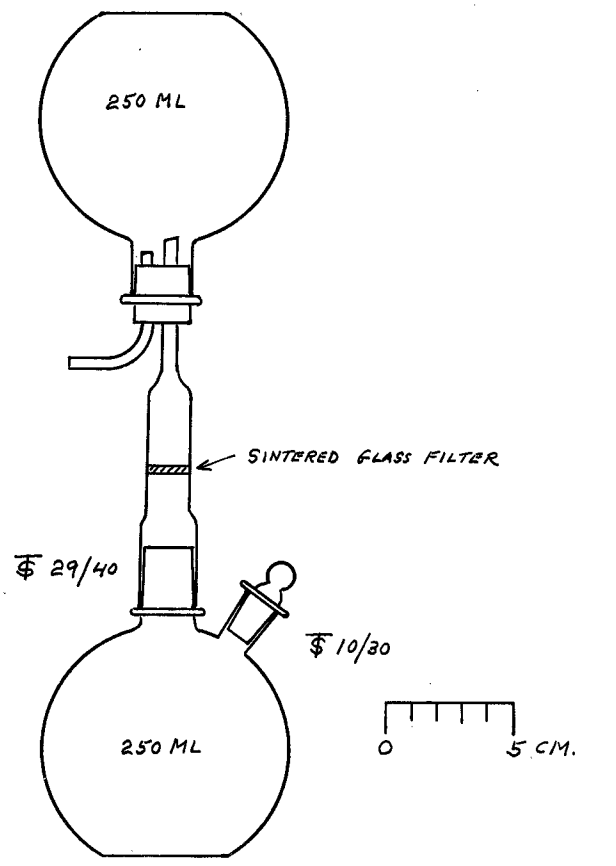

FIG. 1. Apparatus employed to provide an inert atmosphere in the determination of free fatty acid or free caustic alkali and unsaponified material in soap.