The Use of the Polarograph to Distinguish Between the Peroxide Structures in Oxidized Fats[1,2]

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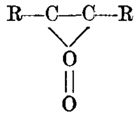
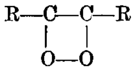
A polarographic method for the determination of peroxides has been applied to fats and related esters. The data presented show that at least three different peroxide structures are present in early stages of oxidation and persist during extensive autoxidation. The peroxides of lard, methyl oleate, and methyl linoleate cannot be distinguished by the polarographic method used in this investigation. Autoxidation of lard at 100°C. is shown to give reduction curves which are much different from those obtained when lard is autoxidized at 45°C. Preliminary work has indicated that the chromatographic technique of Dugan et al. does not separate the peroxide structures which are determined polarographically.

THE term "peroxide" as commonly applied to fats and oils is understood to include all autoxidation products which are capable of oxidizing iodide ions to iodine. It has been suggested that several types of structures which would react in this manner occur in oxidized fats. The development of the theories concerning the mechanism by which atmospheric oxygen reacts with fats and the structures which are formed has been reviewed recently by Markley (6). The proposed structures include peroxides of the following types:

I
R—O—O—R

II

$$\begin{array}{c}\mathrm{R{-}C{-}C{-}R}\\ \backslash\,/\\ \mathrm{O}\\ \|\\ \mathrm{O}\end{array}$$

III

$$\begin{array}{c}\mathrm{R{-}C{-}C{-}R}\\ |\quad\;|\\ \mathrm{O{-}O}\end{array}$$

IV
R—O—O—H

There is ample reason to believe that the autoxidation products of fats include more than one type of peroxide structure and that in many cases several isomeric structures of a given type co-exist in an oxidation mixture. Staudinger (7) published some of the earliest evidence showing the existence of peroxides which differ in stability. Staudinger reported that one of the peroxides which could be obtained from autoxidized asymdiphenylethene exploded at 40-50°C. while another peroxide which was isolated was stable under similar conditions. He proposed that the unstable compound of unknown structure (II) be called a "mol-oxide" and that the relatively stable compound whose structure was believed to be a four-membered ring (III) be called a peroxide.

Recent work has shown that hydroperoxides (IV) are the structures which predominate in autoxidized fats. Farmer (2) and his co-workers have shown that ordinarily atmospheric oxygen adds to the carbon adjacent to the double bond without destroying the unsaturation. Compounds of this type have been isolated by Farmer (3) and also by Swift *et al.* (8). The products isolated from autoxidized methyl oleate consisted of a mixture of the two possible isomeric hydroperoxides. In polyene fatty acids there are several possible isomeric hydroperoxides for each fatty acid. Absorption spectral data indicate that the conjugated isomers which arise as a result of resonance are present in largest amounts. It seems possible that the different isomers may vary in reactivity as well as in concentration depending upon the position of the hydroperoxide group with respect to the double bonds.

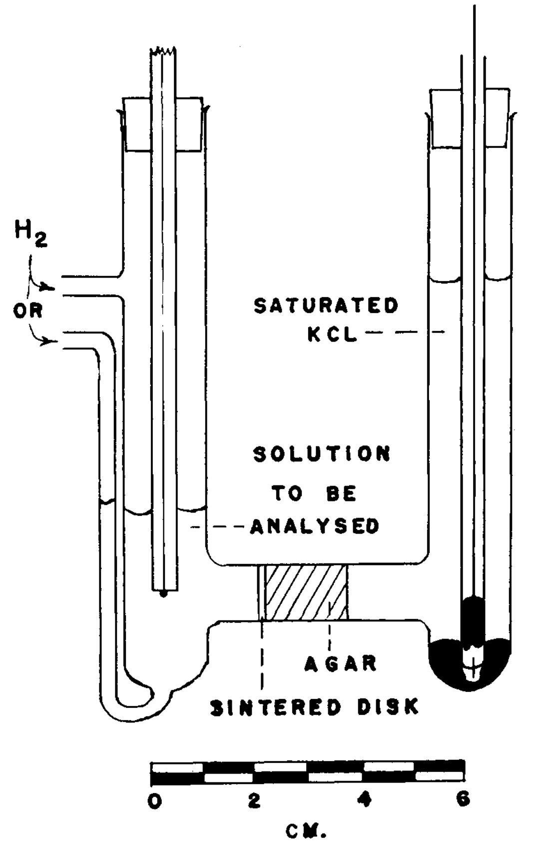


FIG. 1. Electrolysis Cell

In addition to Staudinger's work there is other evidence which indicates that peroxide structures of varying reactivity are often present in fat autoxidation products. For example, it is known that some fatty peroxides react much more slowly with iodides than do others. Consequently the reaction time used in the determining fat peroxides is varied somewhat, depending upon which fatty acids or esters have been oxidized and the conditions under which the autoxidation occurred. It is also known that different fats become organoleptically rancid at different peroxide values. This suggests that some peroxides decompose more readily than others to give the secondary products which are responsible for organoleptic rancidity.

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The relative stability or reactivity of individual peroxide structures is not known with certainty, largely because there has been no analytical procedure available which determined individual fat peroxide structures in the presence of other fat peroxides. It seemed possible that the polarograph might be capable of selectively reducing fat peroxides. Since the polarographic half-wave potential is characteristic of the particular substance being reduced and the wave height is proportional to the concentration of the reducible substance, it seemed that the polarograph might indicate which structures are present and the amount of each.

Experimental

Polarographic Equipment and Procedure: Current-voltage curves were obtained with a Leeds Northrop Electrochemograph, which is a continuous recording instrument. The *m* and *t* values for the capillary were determined at 25°C. with an open electrical circuit and the tip dipping into a 0.3 M solution of lithium chloride in a mixture of equal volumes of absolute methanol and benzene. The values of *m* and *t* were 4.04 mg. per sec. and 2.00 sec. respectively. Therefore $m^{2/3}\ t^{1/6}$ was 2.84 $mg.^{2/3}\ t^{-1/2}$. The electrolysis cell used was a saturated calomel H-cell similar to that described by Lingane and Laitinen (5) (Figure 1). The current-voltage curves shown in the figures were corrected for residual current.

Three supporting electrolytes which varied widely with respect to acidity were used in this investigation. The strongly acid electrolyte used was a 0.2 M solution of methyl hydrogen sulfate. An essentially neutral supporting electrolyte was provided by a 0.3 M solution of lithium chloride. The alkaline solution used was a 0.1 M solution of lithium methoxide. The solvent used for all three supporting electrolytes was a mixture of equal volumes of absolute methanol and benzene. Details concerning the equipment, reagents, and procedure are given by Lewis, Quackenbush, and DeVries (4). Briefly, the procedure was as follows: 10 ml. of supporting electrolyte solution was measured into the cathode compartment of the H-cell. The cell was placed in a 25°C. bath, and the dropping mercury electrode was lowered into the supporting electrolyte solution. Oxygen-free nitrogen was bubbled rapidly through the solution for a minute before the 10 to 200 mg. sample of oil to be analyzed was added to the supporting electrolyte solution from a dropper. The marine barometer tubing, which was used for the dropping mercury electrode, carried a cork which was then lowered until it fitted loosely in the top of the H-cell. Nitrogen was bubbled through the solution for two additional minutes before the polarogram was begun. Nitrogen was passed over the surface of the electrolyte solution during the entire determination since even traces of oxygen interfered seriously with these determinations.

Chemical Method for the Determination of Peroxides: Peroxide values were determined by a method which combined various modifications of the Wheeler (9) method that have been worked out in several different laboratories. A 125-ml. suction flask was fitted with a rubber stopper which carried a 60-ml. separatory funnel. The side arm of the flask was connected to a three-way stopcock which was connected to a water aspirator and a cylinder of oxygen-free nitrogen. The flask could thus be evacuated or filled with nitrogen by simply turning the stopcock. To carry out an analysis a 0.2-0.5 g. sample was measured into the flask. The flask was then evacuated for at least 5 minutes, during which time 10 ml. of a 1:2 chloroform-acetic acid mixture and 1 ml. of a saturated methanol solution of potassium iodide was measured into the separatory funnel. Oxygen-free nitrogen was passed through the flask and bubbled out through the solution in the separatory funnel for five minutes. At the end of this period the solution in the separatory funnel was introduced into the flask by firmly inserting the glass stopper into the top of the separatory funnel while the nitrogen was still bubbling through the solution. This procedure eliminated oxygen from the reagents and the atmosphere over the reaction mixture. The reaction was allowed to proceed 30 minutes at room temperature. Fifteen ml. of distilled water and a few drops of starch solution were added and the liberated iodine was titrated with 0.01 normal sodium thiosulfate.

Polarograms of Oxidized Fatty Esters: Methyl oleate was prepared from olive oil by the method of Wheeler *et al.* (10). It was autoxidized at 40°C. by rapidly bubbling dry air through 10 g. of the sample in a 15-mm. test tube which was immersed in a constant temperature water bath. Peroxide values and current-voltage curves were obtained at the beginning of the experiment and at frequent intervals thereafter. Typical current-voltage curves for oxidized methyl oleate are shown in Figure 2. Similar curves were obtained when either methyl linoleate or lard was autoxidized under the same conditions. In each case three waves were obtained in neutral or acid electrolyte solutions and two waves in alkaline solution. This demonstrated that at least three reducible structures exist in oxidized fats. The position of the waves in the current-voltage curves appeared to be the same for all three substrates. However, the exact position of the small wave which appeared at about —0.12 v. is somewhat in doubt because the supporting electrolyte solution was oxidized at the dropping mercury electrode at potentials which were less negative than —0.12 v.

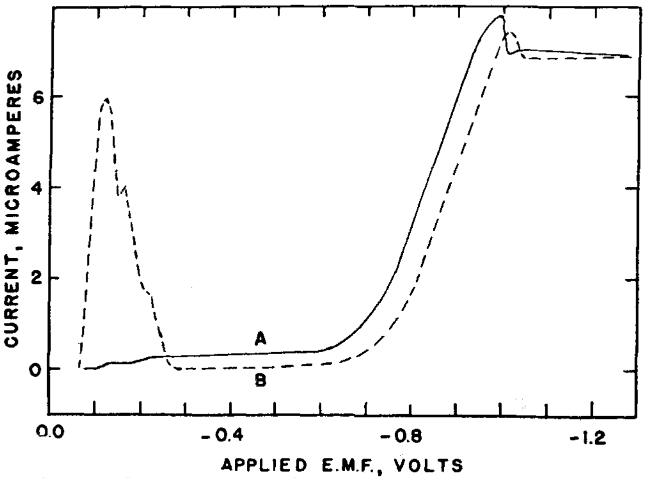

FIG. 2. Current-voltage Curves of Methyl Oleate Oxidized to a Peroxide Value of 35. Supporting electrolytes: (A) 0.3 M lithium chloride, (B) 0.1 M lithium methoxide. Concentration of methyl oleate = 2%.

The three waves are evidently not all due to the same structure, for two reasons. The first reason is that the ratio of the height of one of the waves to the height of either of the other waves was not a constant.

The ratio varied widely depending upon the degree of oxidation of the substrate. If two of the waves were due to the same peroxide structure, the ratio of the heights of the two waves would be a constant. The second reason is that if one electron was involved in the electrode reactions producing either of the small waves, then about 50 electrons would have been involved in the electrode reaction which produced the large wave if the two waves were produced by the same substance. It is unlikely that an electrode reaction would involve so many electrons.

Peroxide Nature of Reducible Substances: Two independent types of evidence show that the waves in Figure 2 are actually due to peroxides. One type of evidence is given in the curves of Figure 3. The upper curve was obtained with lard oxidized to a peroxide value of 48. The lower curve was obtained after the peroxides had been reduced. The reduction was accomplished by treating the lard with potassium iodide in chloroform-acetic acid solution according to the Wheeler procedure (9) for the determination of peroxides. The small wave in the lower curve occurred at a higher potential than the large wave in the upper curve. Therefore, the wave in the lower curve was due to something formed during the reduction of the lard or to a contaminant. Similar results obtained with the acidic and alkaline supporting electrolytes showed that the waves obtained in these solutions were also due to peroxides.

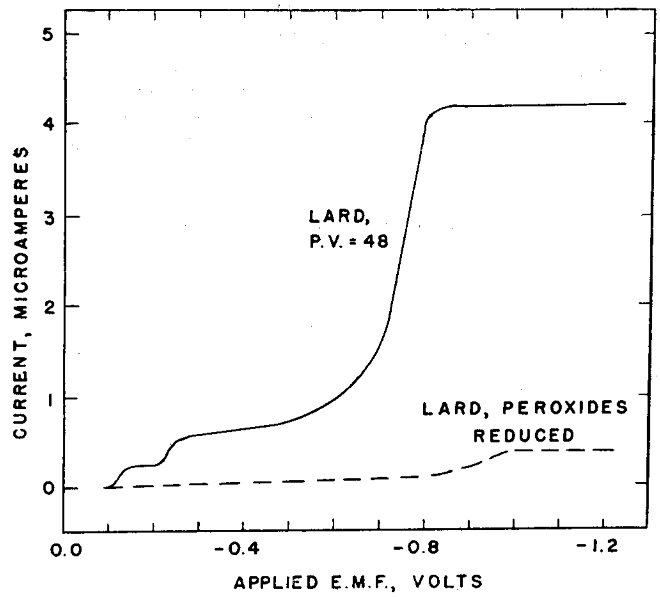

FIG. 3. Current-voltage Curves of Oxidized Lard, Before and After Reduction of Peroxides by the Wheeler Method. The concentration of lard in the 0.3 M lithium chloride supporting electrolyte was 1%.

The second type of evidence indicating that all three waves were due to peroxide structures is that no waves were obtained until peroxides were detectable by chemical methods, but all three waves were evident at peroxide values of one or two even when the autoxidation was carried out at 0°C. It is unlikely that in the early stages of oxidation peroxide decomposition at 0°C. would give rise to significant quantities of secondary products which might have been determined polarographically.

The Relation of Peroxide Number to Wave Height: The relation which exists between peroxide value and wave height is shown in Figure 4. On the vertical axis is plotted the height of the waves obtained when 1% solutions of oxidized methyl linoleate in the lithium chloride supporting electrolyte was analyzed. If the polarograph and the chemical methods determined the same structures, a linear relation would exist. The lower line which is nearly parallel to the horizontal axis of Figure 4 shows the relation between peroxide value and height of the first small wave at —0.15 volts. The broken line indicates the height of the second small wave measured at —0.30 v. A linear relation appeared to exist between the heights of these two small waves and peroxide values. However, the precision of measurement of the height of the small waves was low. Consequently, deviations from linearity would not have been detected unless the deviations were rather large. A linear relation appeared to exist between the peroxide value and the wave height measured at —1.50 volts until the peroxide value reached 275 or 300. Thereafter, the slope of the line decreased. This indicated that the chemical method determined one or more structures which the polarographic method did not.

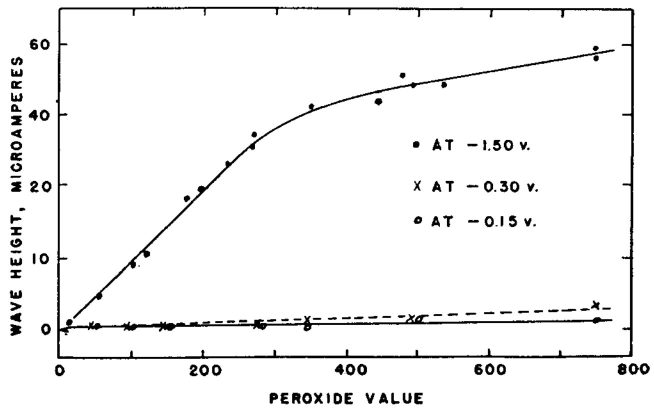

FIG. 4. The Relation Between Peroxide Value and Wave Height. The data were obtained from methyl linoleate oxidized at 40°C. The concentration of methyl linoleate in the 0.3 M lithium chloride was 1%.

It is evident that the peroxide structures represented by the two small waves do not accumulate to any appreciable extent. Until the peroxide value reaches 25 or more, it is difficult to measure the height of these two small waves with reasonable precision, but extrapolation of the curve from higher peroxide values toward the lower values indicates that the structures represented by the first two waves accumulated more rapidly in very early stages of autoxidation than later. Since these structures accumulated more rapidly at the beginning of the oxidation and since the structures never constituted more than a few per cent of the total peroxides it seems probable that they were structures of low stability.

Changes Occurring in Lard Autoxidation Products During Extensive Autoxidation: The changes which occurred in the reduction curves of lard as it was progressively autoxidized at 100°C. are shown in Figure 5. It is evident that there are both qualitative and quantitative changes in lard peroxides during the course of oxidation at 100°C. The three rather specific waves which first appeared gradually gave way to a more general reduction. This suggests the formation of a group of reducible compounds with half-wave potentials which were very close together. A quantitative change of significance was that the pro-

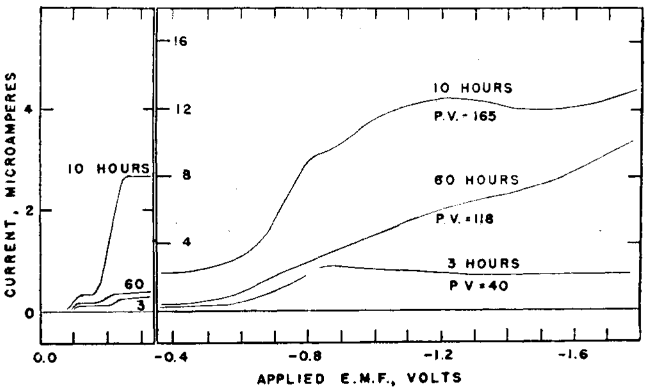

FIG. 5. Typical Current-voltage Curves of Lard Progressively Autoxidized at 100°C. The concentration of lard in the 0.3 M lithium chloride supporting electrolyte was 0.5%.

portion of the peroxide structure represented by the second wave was comparatively high at the end of 10 hours. The peroxide value at this time was 165. After 60 hours the peroxide value had dropped only to 118, but a very large decrease occurred in the height of the second wave. This is another indication that the peroxide structures represented by the first and second waves are rather unstable structures. However, the stability of these structures is far greater than the stability of free radicals of the type which might be present in oxidizing fats. The concentrations of each of the structures represented by the first two waves is of the order of magnitude of .001 M in a fat oxidized to a peroxide value of 100. This concentration is far greater than the concentration of free radicals which would be expected in the free radical oxidative chain reaction mechanism proposed by Farmer and others.

The Effect of Temperature on Autoxidation Products: It is well known that the oxidation products formed at temperatures of 100°C. or over are different from the autoxidation products formed at room temperatures. In the past, evidence of this fact has been largely organoleptic. Recently ultraviolet absorption spectra have given additional evidence that this is true. Polarographic evidence that the products formed from lard during autoxidation at 100°C. are much different from the products formed at 45° C. is given in Figure 6. The 0.3 M lithium chloride supporting electrolyte was used for both curves. Both samples of lard were oxidized until they passed through a maximum peroxide value of over 200. At the time the curves were obtained the peroxide values of the 100° and 45°C. samples had fallen to 112 and 72 respectively. The differences shown were also evident, although to a lesser degree, in earlier stages of oxidation.

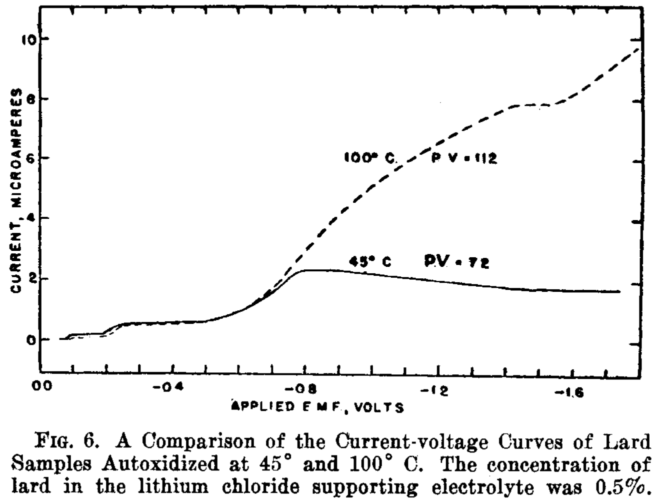

FIG. 6. A Comparison of the Current-voltage Curves of Lard Samples Autoxidized at 45° and 100° C. The concentration of lard in the lithium chloride supporting electrolyte was 0.5%.

Adsorption of Oxidation Products: Preliminary attempts at chromatographic separation of autoxidation products of methyl linoleate which are determined polarographically have not been successful in this laboratory. Dugan *et al.* (1) have shown that peroxides can be removed from oxidized methyl linoleate by adsorption on a column of sodium aluminum silicate adsorbent and that it is possible to divide the column into sections from which distinctly different oxidation products can be eluted. Spectral evidence indicated that the most highly oxidized molecules were adsorbed near the top and that the least oxidized products were adsorbed near the bottom of the column. They postulated that the material eluted from the bottom section represented the primary oxidation product of methyl linoleate. The lower curve of Figure 7 was obtained from an oxidized sample of methyl linoleate which had been adsorbed on a sodium aluminum silicate column and eluted from the bottom section of the column with alcohol. It does not differ from the curves of the original sample of oxidized methyl linoleate shown by the top curve except with respect to the concentration of peroxides represented. The curves shown in Figure 7 were obtained with the lithium methoxide electrolyte solution. Results obtained with the lithium chloride and with the methyl hydrogen sulfate electrolytes also showed that no appreciable concentration of any one of the structures determined polarographically was effected without a corresponding concentration of the other structures.

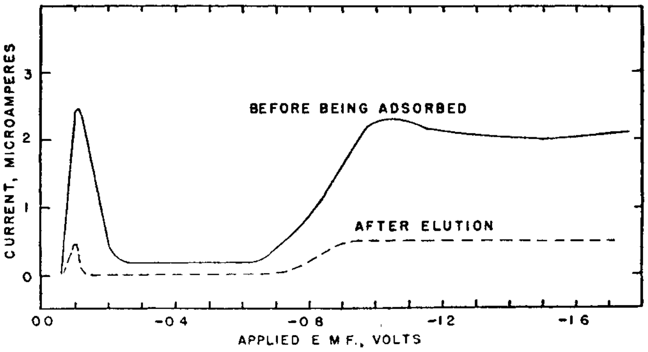

FIG. 7. Current-voltage Curves of Oxidized Methyl Linoleate Before Adsorption and After Elution from Sodium Aluminum Silicate. The concentration of methyl linoleate in the 0.1 M lithium methoxide supporting electrolyte was 0.5%.

Autoxidation of methyl linoleate which was adsorbed on the sodium aluminum silicate adsorbent gave rise to an abnormal autoxidation product. During some of the adsorption experiments the column was allowed to run dry and air was drawn through the column for a few minutes. The column was extruded, cut into sections and eluted as before. This time an additional wave was obtained from the bottom section of adsorbent which began at —1.5 volts when the lithium methoxide supporting electrolyte was used. The wave at —1.5 volts was not obtained in any experiment in which exposure of the adsorbed oxidized methyl linoleate was kept to a minimum.

Summary

A polarographic method for the determination of peroxides has been applied to fats and related esters. The data presented show that at least three different peroxide structures are present in early stages of oxidation and persist during extensive autoxidation. The peroxides of lard, methyl oleate, and methyl linoleate cannot be distinguished by the polarographic method used in this investigation. Autoxidation of lard at 100°C. is shown to give reduction curves which are much different than those obtained when lard is autoxidized at 45°C. Preliminary work has indicated that the chromatographic technique of Dugan *et al.* does not separate the peroxide structures which are determined polarographically.

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Sodium Palmitate in Organic Liquids as Studied by X-Ray Diffraction[1]

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SOAPS in organic liquids have been studied by a number of investigators using various physical-chemical methods.[2] A visual study of the phase behavior of sodium palmitate in various organic liquids was made several years ago in this laboratory (1, 2). This is now followed by an examination of some of the same systems by x-ray diffraction. It is found that at room temperature these systems consist of: 1. the organic liquid; 2. soap curd fibers of one of several types of gamma sodium palmitate; and 3. usually a second crystalline component of unknown composition.

Experimental Methods

The materials used were the original systems prepared for the previous study (1, 2). These sealed tubes had remained undisturbed for several years and so are presumed to be at equilibrium. Samples were removed from them, packed in thin-walled Pyrex capillaries, and mounted in the x-ray beam by a method previously described (3). Care was taken to avoid loss of organic liquid during the loading of the capillary.

The source of x-radiation was a General Electric XRD-1 unit having a tube with copper target and beryllium windows. The radiation was filtered through nickel foil and collimated through guarded pinholes 0.010 inches in diameter and placed 12.5 cm. apart. For the side spacings, a sample to film distance of 50 mm. was used, but for the long spacings this distance was increased to 100 mm. These distances were checked by the 2.814 Å line of sodium chloride.

Results

The x-ray diffraction photographs of these systems are similar to the usual powder photographs produced by solid soaps. They consist of circles or lines corresponding to several orders of a "long spacing" (or spacings, if more than one phase is present) together with lines corresponding to "side spacings." Superimposed on the latter are several haloes due to the organic liquid component of the systems; these haloes tend to obscure some of the side spacings in several of the photographs, in particular those of samples containing the least amount of soap.

Since x-ray powder photographs of heterogeneous mixtures consist of a superposition of the patterns of the individual components, the evaluation of the photographs of the systems studied consisted of comparing the various lines or circles with those of known crystalline substances that might be present. On this basis a few generalizations can be made on all of the systems other than those in cetyl alcohol or palmitic acid as the other organic compound.

In all cases the longer spacings can be ascribed to the various orders of either one or two long Bragg spacings. By far the strongest is that due to the "major" crystalline component present in all photographs (40.5 ± 0.5 Å) which does not change with either the second organic compound present or the concentration of the soap. The weaker long spacing, due to a "minor crystalline component," varies with both concentration and organic liquid and so is not due to a specific chemical substance. It is not present in the photographs of all systems, particularly those containing only a small amount of soap. This may be due to either insufficient material to produce a pattern or else to its complete absence.

For all systems there is one strong "side spacing" which remains constant at 4.71 Å. The other side spacings apparently vary somewhat with the organic liquid but not with concentration. However, these side spacings can be placed in three different groups, as can be seen by a comparison of the data in Tables I, II, and III. The systems more dilute in soap lack the weaker lines, which is to be expected.

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[1] This investigation was conducted partially under contract with the Office of Naval Research under the supervision of Prof. J. W. McBain.

[2] For a recent review of this subject see M. Prasad, G. S. Hattiangdi, and B. K. Wagle, J. Coll. Sci., *2*, 467 (1947).